Linezolid tissue penetration and serum activity against strains of methicillin-resistant Staphylococcus aureus with reduced vancomycin susceptibility in diabetic patients with foot infections

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Objectives: Linezolid soft tissue penetration and serum antimicrobial activity were analysed in six patients with peripheral vascular disease and severe diabetic foot infections requiring surgical intervention.

Methods: Blood draws (1, 3, 6, 9 and 12 h after initiation of a 1 h infusion) and a viable soft tissue sample at the site of infection were obtained in patients receiving linezolid (600 mg every 12 h) on the day of surgery. Concentrations of linezolid were determined by HPLC in both tissue (pre-treated with tissue lysis buffer) and serum. In addition, serum inhibitory and bactericidal activity (dilution titres 1:2–1:32) of linezolid was determined in these patients against strains of methicillin-resistant Staphylococcus aureus (MRSA) with reduced susceptibility to vancomycin (vancomycin MICs = 2, 4, 8, 256 and >256 mg/L).

Results: Linezolid concentrations in tissue were found to be 51% (range, 18% to 78%) of simultaneous serum concentrations. Rapid (1 h) and prolonged (12 h) inhibitory activity (titres ≥1:2) was observed for linezolid against each of the study isolates. Furthermore, bactericidal activity (titres ≥1:2) was observed for at least 6 h (50% of the dosing interval) against four of these five strains.

Conclusions: These findings suggest that linezolid could be effective in the treatment of multidrug-resistant MRSA even when concentrations at the infection site are diminished due to impaired blood flow.

Keywords: pharmacokinetics, pharmacodynamics, diabetic foot infections

Introduction

The treatment of diabetic foot infections is complex and complicated by several risk factors that include peripheral vascular disease, autonomic and sensory neuropathy, and hyperglycaemia.1 Antibiotic selection initially involves decisions concerning the severity of the infection, the route of therapy, the spectrum of organisms to be covered and potential adverse effects.2 Another key to successful antibiotic therapy is achieving therapeutic drug concentrations at the site of infection.3 When peripheral vascular disease is present, therapeutic antibiotic concentrations in infected tissues may not be achieved despite adequate serum levels.4

An antibiotic regimen for diabetic foot infections should generally include an agent active against Staphylococcus aureus and β-haemolytic streptococci. More chronic wounds may need extended coverage that includes Gram-negative bacilli, Enterococcus species and obligate anaerobes.5,6 Methicillin-resistant strains of S. aureus (MRSA) have previously been isolated mainly from hospitalized patients, but community-associated cases are now common.7 Moreover, strains with reduced susceptibility to vancomycin have been isolated in diabetic patients with foot infections.6

Linezolid has been shown to have clinical utility in the treatment of diabetic patients with foot infections.7 It possesses excellent in vitro activity against Gram-positive bacteria including MRSA and vancomycin-resistant enterococci, although most Gram-negative organisms are resistant to linezolid.8 Furthermore, this agent has demonstrated good penetration into bone, joints, muscle and soft tissues in various patient populations.9–12 In this paper, we describe tissue and serum pharmacokinetics of linezolid in diabetic patients with foot infections requiring surgical intervention.
study, we further investigated the serum antibacterial activity and tissue penetration of linezolid in diabetic patients with severe foot infections requiring surgical intervention.

Methods

Subjects

Patients admitted to the hospital surgical service with severe diabetic foot infections were enrolled into this study. In addition, patients receiving antibiotic treatment with only intravenous linezolid (600 mg every 12 h) were eligible to participate in this trial. Each subject gave written informed consent that was approved by the hospital research review committee before entry into this investigation.

Serum and tissue samples

Blood samples were obtained on the day of surgery from each patient at 1, 3, 6, 9 and 12 h after the beginning of a 1 h intravenous infusion of linezolid. A blood sample was also obtained during surgery. Following centrifugation, serum samples were aliquotted and stored at −70°C until the time of analysis. The concentrations of linezolid in sera were measured by a validated HPLC assay (Dr Peloquín, National Jewish Medical and Research Center). The serum curve for linezolid ranged from 0.5 to 30 mg/L. The percent coefficient of variation of a single standard concentration was 0.7%, and the overall validation precision across all standards was 1.0% to 4.4%.

A viable soft tissue sample was obtained from patients at their amputation site. These tissue specimens were wiped gently with dry gauze, placed into a pre-weighed vial and stored at −70°C until the time of analysis. For analysis, tissue samples were removed and sliced into smaller pieces (2–3 mm). They were then placed into separate tube and weighed. Samples were then treated with 0.5 mL of methanol/water (1:1) and vortex mixed for 30 s. Extracted tissue samples were treated with 0.5 mL of tissue lysis buffer (Buffer ATL; Qiagen, Hilden, Germany) vortexed for 30 s and then placed into a 50°C hot water bath for 30 min. The concentration of linezolid from these samples was determined using a modification of the serum HPLC method. The recovery of linezolid from Buffer ATL-treated samples was found to be 96% to 103% from previous analyses.

Bacterial isolates

Well-defined isolates of MRSA were obtained from the National Institutes of Health through the NARSA (Network on Antimicrobial Resistance in S. aureus) programme. The MICs of linezolid and vancomycin were determined by microdilution methods according to the CLSI (formerly the NCCLS). To ensure the stability of vancomycin-resistant strains, agar medium containing 4–6 mg/L of vancomycin was used for subculturing.

Inhibitory and bactericidal titres from sera collected from our patients were determined against these strains of MRSA according to CLSI methodology. Serum was diluted with Mueller–Hinton broth and each determination was performed in duplicate. Wells with no visible growth and the first growth well were subcultured to supplemented Mueller–Hinton agar plates that were incubated for 2 days prior to counting colonies. Isolates were tested against serum collected at each time period for all subjects. The bacteriostatic titre-in-serum endpoint was determined as the highest dilution with no visible growth, and the bactericidal titre-in-serum endpoint was determined as the highest dilution of serum yielding 99.9% killing. The median and geometric mean bacteriostatic and bactericidal titres at each time period for these patients were calculated and used to determine serum inhibitory and bactericidal activity.

Pharmacokinetic analysis

An estimate of pharmacokinetic parameters from these serum samples was derived by non-compartmental analysis using the line of best fit for half-life and the linear trapezoidal rule for AUC\textsubscript{0–12} (WinNon-Lin 4.0, Pharsight, Mountain View, CA, USA) based upon the assumption of steady-state and stable pharmacokinetics.

Results

Six patients (five men) with long-term diabetes were enrolled into this study. These subjects had an age range of 42–81 years (mean, 61 years) and total body weights of 50–127 kg (mean, 98 kg). Each of these patients had peripheral vascular disease with diminished blood flow, documented from previous vascular studies and chronic foot infections requiring partial or complete amputation. Two patients had chronic renal insufficiency but none had liver disease. Serum glucose was controlled by insulin in each of these subjects during this study. All patients did well following surgery and were discharged from the hospital.

The pharmacokinetic parameters for linezolid in serum after multiple doses in these patients are given in Table 1. Tissue samples were obtained during surgery at 1–9 h after the initiation of their linezolid infusion. Linezolid concentrations in these soft tissue specimens ranged from 18% to 78% (mean, 51%) of their simultaneous serum levels (Table 2).

Sera from each patient were tested against five MRSA isolates with reduced susceptibility to vancomycin. The MIC for linezolid was 2 mg/L against all but one of these strains (Table 3). The median serum inhibitory activities of linezolid were similar against each of these bacteria. Linezolid was found to exhibit rapid (1 h) and prolonged (12 h) inhibitory activity against each strain (Table 4). Median bactericidal activity was observed for at least 6 h (20/24 patients) against four of the five strains (Table 4). No bactericidal activity was observed against the MRSA VRS3 isolate.

Table 1. Serum pharmacokinetics of linezolid in the study subjects

| Parameter            | Mean ± SD |
|----------------------|-----------|
| $C_{\text{max}}$ (mg/L) | 14.7 ± 6.1 |
| AUC\textsubscript{0–12} (mg·h/L) | 114 ± 50 |
| $t_{1/2}$ (h) | 5.5 ± 2.8 |
| $V_{ss}$ (L/kg) | 0.44 ± 0.11 |

$C_{\text{max}}$, maximum concentration of linezolid in serum; AUC, area under the concentration–time curve; $t_{1/2}$, half-life; $V_{ss}$, volume of distribution at steady-state.
Linezolid tissue penetration

Table 2. Linezolid concentrations in serum and soft tissue

| Patient | Sampling time (h) | Linezolid concentrations (mg/L) | Serum (mg/L) | Tissue (mg/kg) |
|---------|------------------|---------------------------------|--------------|---------------|
| 1       | 9                |                                 | 14.4         | 11.3          |
| 2       | 9                |                                 | 11.2         | 5.2           |
| 3       | 6                |                                 | 7.1          | 3.9           |
| 4       | 1                |                                 | 22.6         | 10.7          |
| 5       | 3                |                                 | 8.7          | 1.6           |
| 6       | 6                |                                 | 7.5          | 3.7           |
| Mean    |                  |                                 | 11.9         | 6.1           |
| SD      |                  |                                 | 5.9          | 3.6           |

Discussion

Linezolid is an oxazolidinone antimicrobial (MW ~ 337) that is not highly protein-bound (~30%) and has a moderate volume of distribution that approximates total body water.17 Good penetration of linezolid into bone, fat, muscle, soft tissue and inflammatory blister fluid has been demonstrated in healthy volunteers and patients.12,18,19 Following multiple doses of linezolid, in vivo microdialysis studies found subcutaneous adipose tissue-to-plasma ratios of 0.9 in healthy subjects and critically ill patients.12,18 In our patients, the serum pharmacokinetics of linezolid were similar to other investigations, but the mean tissue to serum ratio was only 0.51.18,19 This finding was most likely due to impaired blood flow in our study population. These tissue samples were from the lower extremity in patients with peripheral vascular insufficiency at the infection (sample) site.

Of more importance than the actual tissue concentration of an antibiotic is its antimicrobial activity at the site of infection.3 In general, linezolid exhibits concentration-independent activity against Gram-positive cocci and the time interval of drug concentration that exceeds the MIC (t > MIC) and the area under concentration–time curve to MIC (AUC/MIC) ratio are predictors of efficacy.17,20 Data from animal models demonstrate that a t > MIC of >40% is a good prognostic indicator for a successful outcome.17 Based upon linezolid’s pharmacokinetic parameters after multiple doses, serum concentrations should remain above the MIC_{90} (90% of strains) for Gram-positive bacteria for ≥40% of its dosing interval, even when these levels are diminished by 50% at the site of the infection.17,21 These pharmacokinetic/pharmacodynamic characterizations have been indirectly supported in clinical trials.22 For example, in diabetic patients with foot infections, the majority with foot ischaemia, linezolid exhibited good clinical cure rates including those patients with deep tissue and bone infections.7

Infections caused by S. aureus isolates with reduced susceptibility to vancomycin are a growing problem and effective alternatives are needed.23 Linezolid has good activity against staphylococci, but exhibits predominately bacteriostatic action in in vitro time–kill experiments.24 In contrast to these observations, we found that serum can potentiate the antimicrobial effect of linezolid with resultant bactericidal activity.16 In this current study, linezolid maintained median inhibitory activity for 12 h (17/30 samples) against each of the study isolates and median bactericidal activity for at least 6 h (50% of the dosing interval) for all but one of these S. aureus isolates. These findings are further supported from an in vitro pharmacodynamic model of

Table 3. In vitro activity of linezolid and vancomycin against MRSA strains

| Isolate | Resistance profile | MICs (mg/L) |
|---------|--------------------|-------------|
|         | MecA VanA           | Vancomycin | Linezolid |
| NRS35   | positive negative   | 2           | 2         |
| NRS23   | positive negative   | 4           | 2         |
| NRS404  | positive negative   | 8           | 2         |
| VRS2    | positive positive   | 256         | 1         |
| VRS3    | positive positive   | >256        | 2         |

*Isolates obtained from the Network on Antimicrobial Resistance in S. aureus (NARSA).

Table 4. Median serum inhibitory and bactericidal titres of linezolid against the MRSA study isolates

| MRSA isolate | Time point (h) | SIT (range) | SIT # | SBT (range) | SBT # |
|--------------|---------------|-------------|-------|-------------|-------|
| NRS35        | 1             | 1:2 (1:2–1:16) | 5/6   | 1:2 (1<1:2–1:8) | 3/6   |
|              | 3             | 1:2 (1:2–1:16) | 6/6   | 1:2 (1<1:2–1:8) | 3/6   |
|              | 6             | 1:2 (1:2–1:16) | 6/6   | 1:2 (1<1:2–1:8) | 3/6   |
|              | 9             | 1:2 (1:2–1:16) | 6/6   | 1:2 (1<1:2–1:8) | 3/6   |
|              | 12            | 1:2 (1:2–1:16) | 4/6   | 1:2 (1<1:2–1:8) | 1/6   |
| NRS23        | 1             | 1:2 (1:2–1:16) | 5/6   | 1:2 (1<1:2–1:8) | 1/6   |
|              | 3             | 1:2 (1:2–1:16) | 6/6   | 1:2 (1<1:2–1:8) | 1/6   |
|              | 6             | 1:2 (1:2–1:16) | 6/6   | 1:2 (1<1:2–1:8) | 1/6   |
|              | 9             | 1:2 (1:2–1:16) | 6/6   | 1:2 (1<1:2–1:8) | 3/6   |
|              | 12            | 1:2 (1:2–1:16) | 3/6   | 1:2 (1<1:2–1:8) | 2/6   |
| NRS404       | 1             | 1:2 (1:2–1:16) | 5/6   | 1:2 (1<1:2–1:8) | 4/6   |
|              | 3             | 1:2 (1:2–1:16) | 6/6   | 1:2 (1<1:2–1:8) | 6/6   |
|              | 6             | 1:2 (1:2–1:16) | 6/6   | 1:2 (1<1:2–1:8) | 6/6   |
|              | 9             | 1:2 (1:2–1:16) | 6/6   | 1:2 (1<1:2–1:8) | 3/6   |
|              | 12            | 1:2 (1:2–1:16) | 3/6   | 1:2 (1<1:2–1:8) | 2/6   |
| VRS2         | 1             | 1:2 (1:2–1:16) | 6/6   | 1:2 (1<1:2–1:8) | 5/6   |
|              | 3             | 1:2 (1:2–1:16) | 6/6   | 1:2 (1<1:2–1:8) | 6/6   |
|              | 6             | 1:2 (1:2–1:16) | 6/6   | 1:2 (1<1:2–1:8) | 6/6   |
|              | 9             | 1:2 (1:2–1:16) | 6/6   | 1:2 (1<1:2–1:8) | 5/6   |
|              | 12            | 1:2 (1:2–1:16) | 4/6   | 1:2 (1<1:2–1:8) | 2/6   |
| VRS3         | 1             | 1:2 (1:2–1:16) | 5/6   | 1:2 (1<1:2–1:8) | 0/6   |
|              | 3             | 1:2 (1:2–1:16) | 6/6   | 1:2 (1<1:2–1:8) | 1/6   |
|              | 6             | 1:2 (1:2–1:16) | 6/6   | 1:2 (1<1:2–1:8) | 0/6   |
|              | 9             | 1:2 (1:2–1:16) | 6/6   | 1:2 (1<1:2–1:8) | 0/6   |
|              | 12            | 1:2 (1:2–1:16) | 3/6   | 1:2 (1<1:2–1:8) | 0/6   |

SIT, serum inhibitory titre; SBT, serum bactericidal titre. #, number of subjects with titres ≥1:2.
simulated endocardial vegetations. In these experiments, linezolid exhibited bactericidal activity during the study duration against two clinical strains of vancomycin-resistant S. aureus. Moreover, Howden et al. found that linezolid was effective in 14/18 (78%) patients, including 4 patients with endocarditis, with serious infections due to MRSA with reduced vancomycin susceptibility.

We believe that our findings have clinical relevance for several reasons. This ex vivo pharmacodynamic model integrates antimicrobial activity with pharmacokinetic parameters in patients. Furthermore, serum inhibitory and bactericidal titres allow for an evaluation of antibacterial activity in the presence of factors such as antibodies, complement, protein binding, as well as actual clinically relevant drug concentrations. In analysing serum bacteriostatic and bactericidal activity, patient sera are diluted serially in broth and incubated with the test strain of bacteria. Moreover, these dilutions can simulate the concentration of drug at the site of infection. In general, our experiments found that linezolid exhibited median bacteriostatic and bactericidal activity in these patients at 1:4 and 1:2 dilutions, respectively, for at least 50% of the dosing interval against four of five MRSA strains tested (Figure 1). These findings suggest that linezolid could be effective in the treatment of staphylococcal infec-

![Figure 1. Median serum inhibitory and bactericidal activity of linezolid against a vancomycin-resistant strain (VRS2) of S. aureus. Open squares, inhibitory; filled triangles, bactericidal.](https://academic.oup.com/jac/article-abstract/60/4/819/712270)

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References

1. Tan MJ, Tan JS. Managing foot infections in patients with diabetes. Infect Med 2006; 23: 168–73.

2. Lipsky BA, Berendt AR, Deery G et al. Diagnosis and treatment of diabetic foot infections. Clin Infect Dis 2004; 39: 885–910.

3. Lipsky BA. Medical treatment of diabetic foot infections. Clin Infect Dis 2004; 39 Suppl 2: S104–14.

4. Muller M, dela Pena A, Derendorf H. Issues in pharmacokinetics and pharmacodynamics of anti-infective agents: distribution in tissue. Antimicrob Agents Chemother 2004; 48: 1441–53.

5. Gerdling DN. Foot infections in diabetic patients: the role of anaerobes. Clin Infect Dis 1995; 20 Suppl 2: S283–8.

6. Howden BP, Ward PB, Charles PGP et al. Treatment outcomes for serious infections caused by methicillin-resistant Staphylococcus aureus with reduced vancomycin susceptibility. Clin Infect Dis 2004; 38: S21–8.

7. Lipsky BA, Itani K, Norden C. Treating foot infections in diabetic patients: a randomized, multicenter, open-label trial of linezolid versus ampicillin-sulbactam/amoxicillin-clavulanate. Clin Infect Dis 2004; 38: 17–24.

8. Ross JE, Fritsche TR, Sader HS et al. Oxazolidinone susceptibility patterns for 2005: international report from the Zyvox® annual appraisal of potency and spectrum study. Int J Antimicrob Agents 2007; 29: 285–301.

9. Rana B, Butcher I, Grigoris P et al. Linezolid penetration into osteo-articular tissues. J Antimicrob Chemother 2002; 50: 747–50.

10. Lovering AM, Zhang J, Bannister GC et al. Penetration of linezolid into bone, fat, muscle and haematoma of patients undergoing routine hip replacement. J Antimicrob Chemother 2002; 50: 73–7.

11. Kutscha-Lissberg F, Hebler V, Muhr G et al. Linezolid penetration into bone and joint tissues infected with methicillin-resistant staphylococci. Antimicrob Agents Chemother 2003; 47: 3964–6.

12. Buerger C, Ploch N, Dehghanyar P et al. Pharmacokinetics of unbound linezolid in plasma and tissue interstitium of critically ill patients after multiple dosing using microdialysis. Antimicrob Agents Chemother 2006; 50: 2455–63.

13. Shaikh ZHA, Peloquin CA, Ericsson CD. Successful treatment of vancomycin-resistant Enterococcus faecium meningitis with linezolid: case report and literature review. Scand J Infect Dis 2001; 33: 375–9.

14. National Committee for Clinical Laboratory Standards. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically—Sixth Edition: Approved Standard M7-A6. NCCLS, Wayne, PA, USA, 2003.

15. National Committee for Clinical Laboratory Standards. Methodology for the Serum Bactericidal Test: Approved Guideline M21-A. NCCLS, Wayne, PA, USA, 1999.

16. Stein GE, Schooley SL, Peloquin CA et al. Pharmacokinetics and pharmacodynamics of linezolid in obese patients with cellulitis. Ann Pharmacother 2005; 39: 427–32.

17. MacGowan AP. Pharmacokinetic and pharmacodynamic profile of linezolid in healthy volunteers and patients with Gram-positive infections. J Antimicrob Chemother 2003; 51 Suppl: i17–25.

18. Dehghanyar P, Buerger C, Zeitlinger M et al. Penetration of linezolid into soft tissues of healthy volunteers after single and multiple doses. Antimicrob Agents Chemother 2005; 49: 2367–71.

19. Gee T, Ellis R, Marshall G et al. Pharmacokinetics and tissue penetration of linezolid following multiple oral doses. Antimicrob Agents Chemother 2001; 45: 1843–4.

20. Andes D, van Ogtrop ML, Peng J et al. In vivo pharmacodynamics of a new oxazolidinone (linezolid). Antimicrob Agents Chemother 2002; 46: 3484–9.

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21. Fung HB, Kirschenbaum HL, Ojofeitimi BO. Linezolid: an oxazolidinone antimicrobial agent. Clin Ther 2001; 23: 356–91.

22. Rayner CR, Forrest A, Meagher AK et al. Clinical pharmacodynamics of linezolid in seriously ill patients treated in a compassionate use programme. Clin Pharmacokinet 2003; 42: 1411–23.

23. Cosgrove SE, Carroll KC, Perl TM. Staphylococcus aureus with reduced susceptibility to vancomycin. Clin Infect Dis 2004; 39: 539–45.

24. French GL. Bactericidal agents in the treatment of MRSA infections—the potential role of daptomycin. J Antimicrob Chemother 2006; 58: 1107–17.

25. Cha R, Brown WJ, Rybak MJ. Bactericidal activities of daptomycin, quinupristin-dalfopristin, and linezolid against vancomycin-resistant Staphylococcus aureus in an in vitro pharmacodynamic model with simulated endocardial vegetations. Antimicrob Agents Chemother 2003; 47: 3960–3.

26. Cockerill FR. Conventional and genetic laboratory tests used to guide antimicrobial therapy. Mayo Clin Proc 1998; 73: 1007–21.

27. Bishop E, Melvani S, Howden BP et al. Good clinical outcomes but high rates of adverse reactions during linezolid therapy for serious infections: a proposed protocol for monitoring therapy in complex patients. Antimicrob Agents Chemother 2006; 50: 1559–602.