Antibacterial activity of linezolid and vancomycin in an in vitro pharmacodynamic model of Gram-positive catheter-related bacteraemia

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Objectives: The aim of this study was to compare the activity of linezolid and vancomycin in an in vitro pharmacodynamic model to assess potential differences in activity against biofilm-embedded organisms.

Methods: Single-lumen central venous catheters colonized with biofilm-embedded Staphylococcus aureus, Staphylococcus epidermidis or vancomycin-resistant Enterococcus faecium (VRE) were treated with simulated clinical dosing regimens of linezolid 600 mg every 12 h or vancomycin 1 g every 12 h in a one-compartment in vitro pharmacodynamic model. Quantitative cultures were sampled through the catheter and peripheral ports over 48 h to dynamically assess changes in the burden of catheter colonization and organism seeding, respectively. At 24 and 48 h catheters were removed, sonicated and cultured for adherent organisms.

Results: Both linezolid and vancomycin suppressed bacterial growth on the catheter and release of S. aureus and S. epidermidis into the model compared with controls (P < 0.05), while linezolid also suppressed counts compared with control and vancomycin versus VRE. Neither agent completely eradicated bacterial colonization of the catheters. MICs for the isolates recovered from the model did not increase over time with linezolid or vancomycin exposure.

Conclusions: Lack of activity against biofilm-embedded organisms appeared to be the primary reason for microbiological failure of both drugs in the model.

Keywords: Staphylococcus aureus, Enterococcus, biofilms

Introduction

Use of central venous catheters may be compromised by an increased risk of bloodstream infections, which are associated with prolonged hospitalization, excessive health-care costs and increased patient mortality.1,2 Pathogens responsible for catheter-related bloodstream infections frequently include coagulase-negative staphylococci, Staphylococcus aureus and Enterococcus spp.1,2 Catheter removal at the first signs of infection may not always be feasible and clinicians may be forced to treat infections in situ with either localized or systemic antibiotic therapy. However, these practices can be associated with significant rates of relapse and high morbidity and mortality.1,2 Several mechanisms, including slowing of bacterial growth, physiochemical interactions of the exopolysaccharide matrix with antibiotics and alterations in the cell wall, may be responsible for suboptimal antibacterial activity.3 An ideal antibacterial agent for treating biofilms-encapsulate infections would have activity against dormant bacteria, accumulate to a high degree inside cells and the biofilm matrix and possess a spectrum of activity against relevant catheter-related bloodstream infection (CRBSI) pathogens.
Linezolid and vancomycin activity in biofilms

The oxazolidinone antibiotic linezolid, through inhibition of protein synthesis by binding to the 50S ribosomal subunit, may have greater activity against dormant bacterial populations than cell-wall-active agents. Linezolid also possesses activity against *Staphylococcus* and *Enterococcus* spp., and high intracellular accumulation. We conducted a study to compare the activity of linezolid versus vancomycin against biofilm-encased Gram-positive bacteria commonly associated with CRBSIs using an *in vitro* pharmacodynamic model.

**Materials and methods**

**Bacterial isolates**

Organisms included *S. aureus* ATCC 29213 and clinical strains of *Staphylococcus epidermidis* and vancomycin-resistant *Enterococcus faecium* (VRE).

**Antibacterial agents**

Linezolid (Pharmacia, Kalamazoo, MI, USA) and vancomycin (Sigma, St Louis, MO, USA) powders were dissolved in 0.85% NaCl on the day of use.

**Susceptibility testing**

MICs were determined using vancomycin and linezolid Etest strips (AB Biodisk North America, Piscataway, NJ, USA). The susceptibility endpoint was read at the intersection of the first completely clear ellipse for vancomycin and first discernable growth-inhibition ellipse for linezolid.

**In vitro catheter model**

Sterile single-lumen central venous catheters (Cook Inc., Bloomington, IN, USA) were aseptically placed into 50 mL conical vials containing 40 mL of human plasma after slowly injecting 3 mL of plasma through each lumen. Catheters were then incubated with shaking at 37°C. After 24 h, catheters were transferred into vials containing 40 mL of cation-supplemented Mueller–Hinton broth and 1 mL of a bacterial suspension (~1.5 × 10⁸ cfu/mL) was injected through the lumen. Catheters were then incubated in broth at 37°C in a shaking incubator for 24 h to allow biofilm formation. Catheters were removed from the broth and slowly rinsed with 10 mL of sterile 0.85% saline and vortexed to remove non-adherent bacteria.

A one-compartment catheter-related model was used to simulate the serum pharmacokinetics of clinical dosing regimens of linezolid (600 mg every 12 h) and vancomycin (1 g every 12 h). The central glass compartment (1000 mL) contained a magnetic stir bar for continuous mixing and sample ports to allow for serial removal of samples and was maintained in a water bath at 37°C. Sterile Mueller–Hinton broth was pumped into the central compartment by a peristaltic pump (Masterflex LS, Vernon Hills, IL, USA) at a fixed rate to simulate the half-lives of linezolid and vancomycin. Two colonized catheters were inserted into the central compartment and the model was allowed to equilibrate for 15 min. To achieve clinical pharmacokinetic parameters and simulate concentrations of antimicrobials injected through catheters, 10 mL of linezolid or vancomycin stock solutions (1.5 and 3.0 mg/mL, respectively) were then infused over 10 min through the catheter lumens. At predetermined time-points samples were acquired via the catheter lumen and peripheral port (to assess release of bacteria from the biofilm into the model), serially diluted and spiral plated (50 μL) for cfu enumeration.

**Pharmacokinetic analysis**

Pharmacokinetics were verified by bioassay. TSA-blood agar plates were flooded with a standardized cell suspension (1 × 10⁶ cfu/mL) of *S. aureus* ATCC 29213 and allowed to dry at ambient temperature. For the vancomycin bioassay, wells were cored into the agar and 200 μL aliquots of a vancomycin solution or unknown sample were pipetted into the wells. For linezolid, sterile cloning cylinders (Corning Life Sciences, Corning, NY, USA) were placed on top of the bacterial lawn prepared as above and 200 μL aliquots of standardized linezolid solution medium or unknown sample from the model were pipetted into the cylinders. The plates were allowed to incubate for 24 h at 37°C before inhibition zones were measured. Each bioassay was performed in triplicate. Peak concentration (Cₘₐₓ), trough concentration (Cₘᵢₙ) and elimination half-life (t₁/₂) were calculated from the concentration–time plots.

**Pharmacodynamic analysis**

Colony count data for samples removed from the model were plotted as a function of time for each isolate/drug regimen tested. Colony counts recovered from samples acquired through the catheter and peripheral ports as well as counts recovered from sonicated catheter cultures were compared by analysis of variance with Tukey’s test for multiple comparisons using Prism software, version 4.0 (GraphPad Software, Inc., San Diego, CA, USA). A *P* value of ≤ 0.05 was considered significant.

**Results**

**Susceptibility testing**

Median MICs of linezolid for *S. aureus* ATCC 29213, *S. epidermidis* and VRE were 1.75, 1.5 and 1.0 mg/L, respectively. Median MICs of vancomycin for *S. aureus*, *S. epidermidis* and VRE were 2.0, 3.0 and > 32 mg/L, respectively. MICs for organisms recovered from the model at 48 h did not differ from the MIC for the starting inocula.

**Pharmacodynamic analysis**

Both linezolid and vancomycin were bacteriostatic against *S. aureus* and *S. epidermidis* with bacterial counts in samples pulled through the catheter and peripheral sampling port exhibiting ≤ 2 log₁₀ cfu reduction from the starting inoculum (Figure 1). Linezolid was also bacteriostatic against VRE. Persistent bacterial release from the biofilm was seen with all isolate/drug combinations, but was reduced to the greatest extent with linezolid against VRE (Figure 1f). Sonicated catheter cultures demonstrated that both linezolid and vancomycin were effective in reducing the number of bacteria from catheters colonized by *S. epidermidis* compared with controls (*P* < 0.05) at both 24 and 48 h (Figure 2). Linezolid was also effective at reducing the number of colonizing *S. aureus* bacteria compared with controls (*P* < 0.05) at 24 and 48 h. No significant differences in catheter cultures were noted for either regimen against VRE; however, there was a trend towards decreased colony counts from sonicated cultures with linezolid (Figure 2). None of the colonized catheters was completely sterilized following exposure to antibacterials.
Pharmacokinetic analysis

Standard curves for both the linezolid and vancomycin bioassays were linear over the concentration range tested ($r^2 = 0.989$ and 0.988, respectively), and intra- and inter-day coefficients of variation were <10%. The lower limit of detection for both vancomycin and linezolid was 2 mg/L. Measured peak, trough and elimination half-life were similar to values achieved clinically for linezolid and vancomycin ($C_{\text{max}}$ 13.3 ± 1.62 and 36.4 ± 3.77 mg/L; $C_{\text{min}}$ 2.90 ± 0.58 and 10.5 ± 0.25 mg/L; $t_{1/2}$ 5.4 ± 1.4 and 6.69 ± 1.29 h, respectively; means ± SD).

Discussion

Results from our in vitro pharmacodynamic catheter model are consistent with clinical observations that currently available antibiotic therapy alone has a limited ability to eradicate biofilm-embedded organisms. While both linezolid and vancomycin prevented further growth of susceptible organisms, neither agent completely sterilized the biofilm-embedded bacteria on a catheter matrix.

Like all in vitro studies, our model is limited in terms of the physiological and environmental factors that could affect
antimicrobial activity in vivo. However, previous studies have suggested that in vitro models similar to the one employed in this study can discriminate between effective and less effective treatment strategies for CRBSI in humans.7,8 While antibiotic penetration into the biofilm was not directly measured in this study, reduced penetration as a means of resistance seems unlikely. Previous studies of linezolid and vancomycin have failed to demonstrate complete eradication of bacteria from endoluminal surfaces, even with high concentrations.9,10 In our study, post-exposure MICs were similar to those measured prior to antibiotic exposure, suggesting a lack of acquired resistance following exposure to linezolid or vancomycin.

The limited activity of vancomycin and linezolid against biofilm-embedded organisms does not preclude their use in CRBSI. Rather, it emphasizes the need for better preventative approaches that limit biofilm formation. It should be recognized that complete eradication of biofilm-embedded organisms in situ by medical therapy alone is unlikely. However, reduction in colonization density or burden through antimicrobial therapy with or without adjunctive strategies could potentially delay the onset or intensity of bacteraemia until the catheter can be removed. Therefore, optimal regimens for salvaging catheters infected with Gram-positive cocci should still be pursued in parallel with more effective preventative strategies.

In conclusion, although both linezolid and vancomycin were able to suppress growth of the Gram-positive organisms tested in the pharmacodynamic model and did reduce colonization density due to S. epidermidis and VRE (linezolid), neither regimen was successful in completely eradicating catheter-related bacterial colonization. These results illustrate the inherent difficulty in sterilizing central venous catheters and reaffirm the need for combined approaches for catheter sterilization, as well as prompt removal of catheters whenever possible in patients with bacteraemia due to Staphylococcus and Enterococcus spp.

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References