Activity of telavancin against heterogeneous vancomycin-intermediate \textit{Staphylococcus aureus} (hVISA) \textit{in vitro} and in an \textit{in vivo} mouse model of bacteraemia

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Objectives: Infections caused by heterogeneous vancomycin-intermediate \textit{Staphylococcus aureus} (hVISA) are associated with high rates of vancomycin treatment failure. Telavancin is a bactericidal lipoglycopeptide active \textit{in vitro} against Gram-positive pathogens including hVISA and vancomycin-intermediate \textit{S. aureus} (VISA). This study characterizes the microbiological activity of telavancin against vancomycin-susceptible \textit{S. aureus} (VSSA), hVISA and VISA strains.

Methods: Reference strains of VSSA, hVISA and VISA were assessed for potential telavancin heteroresistance by population analysis. In addition, the efficacies of telavancin (40 mg/kg subcutaneously every 12 h for 4 days) and vancomycin (110 mg/kg subcutaneously every 12 h for 8 days) were compared in a neutropenic murine model (immunocompromised female non-Swiss albino mice) of bacteraemia caused by hVISA strain Mu3. Blood and spleen bacterial titres were quantified from cohorts of mice euthanized pre-treatment and at 24 h intervals post-treatment for 8 days.

Results: Telavancin was active against all strains of \textit{S. aureus} tested, with MIC values \(<0.5\) mg/L. Population analyses revealed no evidence of subpopulations with reduced susceptibility to telavancin. In the murine bacteraemia model of hVISA infection, all animals were bacteraemic pre-treatment and mortality was 100\% within 16–24 h post-infection in untreated animals. Treatment with telavancin was associated with lower spleen bacterial titres, lower rates of bacteraemia and lower overall mortality than treatment with vancomycin.

Conclusions: These \textit{in vitro} and pre-clinical \textit{in vivo} studies demonstrate that telavancin has the potential to be efficacious in infections caused by hVISA.

Keywords: heteroresistance, glycopeptides, lipoglycopeptides, Mu3, ATCC 700698

Introduction

Methicillin-resistant \textit{Staphylococcus aureus} (MRSA) is an increasingly prevalent pathogen in serious infections. MRSA strains with reduced susceptibility to glycopeptides have emerged and may be associated with vancomycin treatment failure.\(^1\) Reduced susceptibility to glycopeptides in vancomycin-intermediate \textit{S. aureus} (VISA) results from a thickened cell wall and non-productive antimicrobial binding to non-cross-linked D-alanyl-D-alanine residues.\(^2\) Heterogeneous VISA (hVISA) strains, which are susceptible to vancomycin but contain subpopulations of cells with reduced susceptibility (MIC \(>2\) mg/L), appear to be precursors of VISA.\(^3\) Increasing prevalence of hVISA infections means that there is a growing need for alternative therapeutic agents with activity against hVISA.

Telavancin is a once-daily injectable, semi-synthetic lipoglycopeptide antibiotic with bactericidal activity against susceptible Gram-positive organisms including MRSA, hVISA and VISA.\(^2\)\(^-\)\(^5\) The 24 h area under the concentration curve (AUC)/MIC ratio is the pharmacodynamically linked pharmacokinetic parameter for telavancin, as is the case for vancomycin.\(^6\) The antibacterial activity of telavancin against organisms with reduced vancomycin susceptibility is thought to result from enhanced targeting to the membrane-embedded cell wall precursor, lipid II, relative to D-alanyl-D-alanine residues in the mature cell wall.\(^7\)\(^,\)\(^8\)

In the present study, the microbiological activity of telavancin is characterized against vancomycin-susceptible \textit{S. aureus} (VSSA), hVISA and VISA strains. Population analysis profiling (PAP) was used to test for potential telavancin heteroresistance.
In a previous study, we demonstrated that acute dosing (1 day) with telavancin was more effective than vancomycin against an hVISA infection in a neutropenic mouse bacteraemia model. Since hVISA infections are particularly problematic during chronic therapy, we have conducted additional studies to compare the efficacies of repeat dosing with telavancin (4 days) and vancomycin (8 days) in an immunocompromised murine model of bacteraemia caused by the hVISA reference strain, Mu3. We tested the hypothesis that the superior antibacterial activity of telavancin, compared with vancomycin, would translate into similar or more rapid clearance of the infection with a shorter course of therapy.

Materials and methods

Organisms

Three S. aureus reference strains, VSSA (ATCC 29213), Mu3 [ATCC 700698 (hVISA)] and Mu50 [ATCC 700699 (VISA)], were obtained from the American Type Culture Collection (ATCC; Manassas, VA, USA).

MIC determination

MIC values were determined by broth microdilution according to CLSI methodology, using commercially prepared dry-form test panels obtained from Trek Diagnostics (Cleveland, OH, USA).

Detection of heteroresistance

PAP assays to detect heteroresistance were performed as described previously, with the following modifications. Fresh overnight colonies on tryptic soy agar (TSA) plates were suspended to an optical density of 0.5–0.6 at 625 nm (5×10^8–1×10^9 cfu/mL) and diluted in cation adjusted Mueller–Hinton broth (Hardy Diagnostics, Santa Maria, CA, USA) to yield suspensions of 10^6, 10^7, and 10^8 cfu/mL. Aliquots (50 μL) of each cell suspension were spiral plated (WASP2 Spiral Plater; Don Whitley Scientific, Frederick, MD, USA) onto drug-free brain heart infusion (BHI) agar plates and BHI plates containing either vancomycin or telavancin at indicated concentrations. Colonies were counted after incubation at 37°C for 48 h using an automated colony counter (SR92000; ProtoCOL, Frederick, MD, USA). The lower limit of quantification was 400 cfu/mL (2.6 log_{10} cfu/mL). PAPs were generated by plotting cfu/mL against antibiotic concentration using GraphPad Prism (v4.03; GraphPad, Inc., San Diego, CA, USA) and the AUC was calculated. Vancomycin PAP/AUC ratios (AUC of test strain/AUC of Mu3) of <0.9, 0.9–1.3 and >1.3 were used to define VSSA, hVISA and VISA, respectively.

Neutropenic murine bacteraemia model

All studies were conducted with the approval of the Institutional Animal Care and Use Committee at Theravance, Inc. and were executed in an Association for Assessment and Accreditation of Laboratory Animal Care accredited facility. The experimental model of infection and study design was similar to that described previously, with certain modifications. Female non-Swiss albino mice (Harlan, Indianapolis, IN, USA), weighing 18–30 g, were rendered neutropenic with 250 mg/kg cyclophosphamide, intraperitoneally (ip), 4 and 2 days before infection. Neutropenic animals were subsequently inoculated ip with 0.7 mL of inoculum containing 10^7 cfu/mL hVISA strain Mu3. Neutropenia was sustained over 8 days with 125–150 mg/kg cyclophosphamide, ip, administered 2 and 5 days post-infection.

For the vancomycin study, eight groups of neutropenic animals (n=5 per group) were treated with 110 mg/kg vancomycin every 12 h, subcutaneously (sc), starting 4 h post-infection and continuing for 8 days. For the telavancin study, eight groups of animals (n=5 per group) were treated with 40 mg/kg telavancin every 12 h, sc, starting 4 h post-infection and continuing for 4 days. A shorter course of therapy for telavancin was chosen compared with vancomycin, to determine whether its superior microbiological activity would translate to faster clearance of the pathogen. Within each treatment arm, an additional three groups of animals (n=5 per group) received no treatment (controls). Moribund animals were euthanized humanely. At the doses tested, AUC_{0–24} of free telavancin (63 mg·h/L) and vancomycin (130 mg·h/L) in mice approximate human AUC_{0–24} values at intravenous human doses of 10 mg/kg telavancin every 24 h or 1 g of vancomycin every 12 h, respectively. Designated groups of telavancin- and vancomycin-treated animals were euthanized humanely via CO2 asphyxiation at 24 h intervals post-infection up to day 8. Groups of untreated controls were euthanized at 1, 4 (pre-treatment) and 16 h post-infection; no untreated control animals survived beyond this timepoint. Blood was drawn via cardiac puncture and spleens were excised aseptically, weighed and homogenized. Samples were then serially diluted and plated onto TSA plates, which were incubated for 24 h at 37°C and the bacterial colonies counted. Titres were expressed as log_{10} cfu/mL (blood) or log_{10} cfu/g (spleen). The limit of quantification was 1.0 log_{10} cfu/mL and 2.1 log_{10} cfu/g in blood and spleen, respectively.

Results

In vitro susceptibility and assessment of heteroresistance

MICs of telavancin, vancomycin and teicoplanin for the VSSA (ATCC 29213) strain were 0.25, 1 and 0.5 mg/mL, respectively; for the Mu3 strain (hVISA) the MICs were 0.5, 2 and 2 mg/mL, respectively; and for the Mu50 strain (VISA) the MICs were 0.5, 4 and 4 mg/mL, respectively.

Vancomycin PAP assays confirmed the hVISA and VISA phenotypes of strains Mu3 and Mu50, respectively (Figure 1). Growth of resistant subpopulations in both of these isolates was readily detected above the vancomycin susceptibility breakpoint of 2 mg/L. Vancomycin PAP/AUC ratios were 0.6 for ATCC 29213, 1.0 for Mu3 and 2.6 for Mu50. Telavancin PAP assays failed to detect subpopulations with reduced susceptibility (Figure 1); no colony growth was observed at telavancin concentrations >0.5 mg/L.

Efficacy of telavancin and vancomycin in a murine model of hVISA bacteraemia

A cyclophosphamide dosing regimen that produced a sustained reduction in murine neutrophil count over the 8 day post-infection period was employed. The mean blood neutrophil count decreased from 795±105 cells/μL at −4 days pre-infection to 23.3±3.33, 56.7±6.67 and 86.7±8.82 cells/μL at 1, 4 and 7 days post-infection, respectively.

In the vancomycin study, blood titres in untreated controls were 5.20±0.49 log_{10} cfu/mL and 5.93±0.52 log_{10} cfu/mL at 4 h and 16 h post-infection, respectively. On days 5–7, a significant proportion of vancomycin-treated animals (40%–100%) had positive blood cultures. Spleen titres in untreated controls were 7.56±0.39 log_{10} cfu/g and 8.94±0.41 log_{10} cfu/g at 4 h
and 16 h post-infection, respectively. Vancomycin at 110 mg/kg every 12 h decreased spleen Mu3 titres during days 1–3 (P < 0.05 on day 3 versus pre-treatment values). However, cfu counts increased during days 4–7 (P > 0.05 versus pre-treatment values) despite ongoing vancomycin treatment (Figure 2).

In the telavancin study, blood titres in untreated controls were 4.46 ± 0.04 log_{10} cfu/mL and 6.42 ± 0.22 log_{10} cfu/mL at 4 h and 16 h post-infection, respectively. No telavancin-treated animals had positive blood cultures after day 1. Spleen titres in untreated controls were 7.09 ± 0.27 log_{10} cfu/g and 9.31 ± 0.26 log_{10} cfu/g at 4 h and 16 h post-infection, respectively. Four days of treatment with 40 mg/kg telavancin every 12 h produced a sustained and significant reduction (P < 0.05 versus pre-treatment values) in spleen Mu3 titres throughout the 8 day study period (Figure 2).

Mortality was 100% (30/30 animals died) within 16 h post-infection among untreated controls. Three vancomycin-treated animals died (7.5%): two on day 4 and one on day 8. No telavancin-treated animals died over the 8 day study period.

Discussion

Staphylococcal infections caused by hVISA are increasing and their reduced therapeutic responsiveness to vancomycin represents an emerging threat to public health. In the present study, telavancin had MIC values between 0.25 and 0.5 mg/L for reference strains of VSSA, VISA and hVISA. Telavancin PAP of hVISA and VISA strains did not reveal evidence of telavancin heteroresistance. In a neutropenic murine model of hVISA bacteraemia, telavancin (4 days of treatment) was more efficacious than vancomycin (8 days of treatment) in clearing the infection; this was accompanied by a lower mortality rate in telavancin-treated compared with vancomycin-treated animals (0% versus 7.5%).

These preliminary in vitro and in vivo pre-clinical data suggest that telavancin may have clinical utility for the treatment of hVISA infections and further randomized controlled trials are warranted.

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