Efficacy of telavancin in a murine model of pneumonia induced by methicillin-susceptible *Staphylococcus aureus*

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**Objectives:** To assess the efficacy of telavancin, a rapidly bactericidal lipoglycopeptide, and three comparator agents in a murine model of pneumonia induced by methicillin-susceptible *Staphylococcus aureus* (MSSA).

**Methods:** Female Bagg inbred albino c-strain (BALB/c) mice were rendered neutropenic and infected by intranasal inoculation (50 µL) of $10^7$ cfu of *S. aureus* ATCC 29213. Infected mice were then allocated to one of five treatment arms: subcutaneous (sc) telavancin 40 mg/kg every 12 h, sc nafcillin 40 mg/kg every 4 h, sc vancomycin 110 mg/kg every 12 h, intravenous linezolid 80 mg/kg every 12 h or no drug (control group). Test compounds were studied under low and high pre-treatment titre conditions by initiating drug treatment at 4 and 8 h post-inoculation, respectively. Drug doses were calculated to simulate human exposures (area under the curve or $t >$MIC) at therapeutic doses. Lungs were harvested and homogenized 24 and 48 h after inoculation to determine the bacterial titre.

**Results:** At 48 h post-inoculation in the low and high pre-treatment titre groups, respectively, telavancin produced greater reductions (from pre-treatment values) in bacterial burden ($-2.43$ and $-2.32$ log$_{10}$ cfu/g) than nafcillin ($-1.3$ and $-1.8$ log$_{10}$ cfu/g), vancomycin ($-2.9$ and $-2.2$ log$_{10}$ cfu/g) and linezolid ($-0.4$ and $+0.3$ log$_{10}$ cfu/g).

**Conclusions:** These findings support the potential clinical utility of telavancin in the treatment of MSSA pneumonia.

Keywords: mouse, MRSA, lipoglycopeptide, vancomycin, nafcillin

**Introduction**

Nosocomial pneumonia is the second most frequently encountered infection in the healthcare setting and is associated with high morbidity, mortality and use of medical resources. Depending on the diagnostic definition and population sampled, hospital-acquired pneumonia occurs in approximately 5–10 cases per 1000 admissions and accounts for up to 25% of intensive care unit infections. The incidence of ventilator-associated pneumonia is several fold higher in patients admitted to the intensive care unit than that of hospital-acquired pneumonia in the general hospital population. *Staphylococcus aureus* is now the most common Gram-positive organism implicated in nosocomial pneumonia. The clinical status and prognosis of *S. aureus*-associated nosocomial pneumonia do not appear to differ between patients with methicillin-resistant (MRSA) and methicillin-susceptible (MSSA) strains.

Delays in identifying the causative pathogen(s) responsible for nosocomial pneumonia mean that affected patients often require prompt initiation of empirical therapy to achieve clinical cure and to reduce the likelihood of colonization with multidrug-resistant pathogens. Vancomycin and linezolid are recommended empirical and definitive therapies for nosocomial pneumonia, but as only two-thirds of patients obtain a clinical cure after treatment for up to 3 weeks, novel antibacterial drugs are urgently required to improve outcomes.

Telavancin is a novel, rapidly bactericidal lipoglycopeptide antibiotic with a unique, multifunctional mechanism of action that includes inhibition of cell wall peptidoglycan biosynthesis and disruption of the functional integrity of the bacterial membrane. In a previous study, telavancin was shown to be efficacious against MRSA in a neutropenic murine model of pneumonia. As a considerable proportion of nosocomial pneumonia is due to MSSA, the goal of this study was to compare...
the efficacy of telavancin in a neutropenic murine model of MSSA pneumonia with that of nafcillin (considered the treatment of choice in the USA for MSSA infections), vancomycin and linezolid.

Materials and methods

This study was approved by the Institutional Animal Care and Use Committee at Theravance, Inc. For in vitro studies, telavancin (Theravance, Inc., South San Francisco, CA, USA) was prepared as a stock solution of 4 mg/mL in 50% dimethyl sulfoxide, 0.003 M hydrochloric acid. Stock solutions of nafcillin (Sigma-Aldrich, St Louis, MO, USA), vancomycin (Sigma-Aldrich) and linezolid (Pharmacia, Kalamazoo, MI, USA) were prepared according to the recommendations of the CLSI (M100-S16). For in vivo studies, telavancin for injection (250 mg/vial) (Theravance, Inc.) was reconstituted in 5% dextrose in water, nafcillin (Sandoz Inc., Princeton, NJ, USA) was reconstituted in water, and vancomycin (Sigma-Aldrich) and linezolid (Pharmacia) were dissolved in 5% dextrose in water and 25% hydroxypropyl-β-cyclodextrin, respectively. S. aureus ATCC 29213 was obtained from the ATCC (Manassas, VA, USA). MIC values were determined by the broth microdilution methodology according to the CLSI. Bactericidal activity was determined by time–kill assay in 10 mL of Mueller–Hinton broth with a starting inoculum of ~10^6 cfu/mL log-phase cultures. At regular intervals, bacteria were enumerated by plate counting from 100 µL samples. Bactericidal activity was defined as a ≥3 log_10 decrease in the number of cfu/mL within 24 h. The limit of detection was 10 cfu/mL.

Female Bagg inbred albino c-strain (BALB/c) mice (Harlan, Indianapolis, IN, USA), weighing 16–26 g, were rendered neutropenic with intraperitoneal cyclophosphamide (200 mg/kg) at 4 and 2 days prior to MSSA exposure. The neutropenic mice were lightly anaesthetized with isoflurane gas and then held in an upright position to be inoculated. Infection was established by intranasal inhalation (50 µL) of 10^7 cfu of MSSA ATCC 29213 as small droplets. After inoculum inhalation, the animals were placed back in their respective cages for observation, prior to dosing with test compounds.

The antibacterial effects of test compounds were studied under low and high pre-treatment titre conditions by initiating treatment at 4 or 8 h post-inoculation, respectively. Six infected mice per group received subcutaneous (sc) telavancin 40 mg/kg every 12 h, sc nafcillin 40 mg/kg every 4 h, sc vancomycin 110 mg/kg every 12 h, intravenous (iv) linezolid 80 mg/kg every 12 h or no drug (control group). Test doses in mice were calculated to simulate human therapeutic exposures [based on area under the 0–24 h time curve (AUC_0–24) or t > MIC] using mouse pharmacokinetic data for telavancin, nafcillin (Theravance, Inc.; data on file), vancomycin and linezolid. The telavancin dose regimen used produces total (free) drug AUC_0–24 of 747 (52.3) mg·h/L (approximately equivalent to that achieved at a human therapeutic dose of 10 mg/kg, iv, once daily); the nafcillin dose regimen produces t > MIC for 50% of the dosing interval (approximately equivalent to a human therapeutic dose of 2 g, intramuscularly, once every 4 h); the vancomycin dose regimen used produces total (free) drug AUC_0–24 of 225 (130) mg·h/L (approximately equivalent to a human therapeutic dose of 1 g, iv, once every 12 h); and the linezolid dose regimen used produces total (free) drug AUC_0–24 of 160 (112) mg·h/L (approximately equivalent to a human therapeutic dose of 600 mg, iv, once every 12 h).

Animals were euthanized at 24 and 48 h post-inoculation, and the lungs were harvested and homogenized in saline. Serial dilutions of these homogenates were plated onto tryptin-soy agar plates containing 1 mg/L aztreonam (to select for MSSA), incubated overnight at 30°C and read the following day to quantify the numbers of cfu. The lung titre was expressed as log_{10} cfu/g of lung weight. One-way analysis of variance (ANOVA) was used for statistical analysis.

Discussion

The present study has demonstrated that telavancin displays rapid bactericidal activity in vitro and is also efficacious in vivo against MSSA in a murine model of pneumonia. The greater efficacy of telavancin, compared with vancomycin and linezolid, in this MSSA pneumonia model is consistent with previous findings in a similar animal model in which MRSA (ATCC 33591) was the test pathogen. As semi-synthetic penicillins are preferred for the treatment of MSSA infections, it was important to compare the relative efficacy of telavancin with nafcillin in the present study. The data show that telavancin produces significantly greater and more rapid reduction in lung titre than nafcillin. Comparative studies with oxacillin and cloxacinil may be required to determine whether the superior in vivo activity of
Telavancin efficacy in mouse MSSA pneumonia

Figure 1. Time–kill curves for MSSA ATCC 29213 after exposure to telavancin (TLV), nafcillin (NAF), vancomycin (VAN) and linezolid (LZD) at equal multiples of their respective MIC values. The TLV, NAF, VAN and LZD MIC values were 1, 0.5, 1 and 2 mg/L, respectively.

Telavancin is also observed relative to alternate semi-synthetic penicillins.

The superior efficacy of telavancin against MSSA and MRSA in these models may be due to its unique multifunctional mechanism of action comprising inhibition of bacterial cell wall peptidoglycan synthesis and disruption of the functional integrity of the bacterial membrane. Findings from time–kill studies of MRSA have shown that, at 8×MIC, linezolid behaved as a bacteriostatic drug, whereas vancomycin was slowly bactericidal requiring up to 24 h to elicit a ≥3 log₁₀ reduction in inoculum size. In contrast, at the same MIC multiple (8×MIC), telavancin produced a ≥3 log₁₀ reduction in bacterial counts. In the murine MSSA pneumonia model (ATCC 29213), n = 6 at the time of dosing in each group. *P < 0.05 versus corresponding pre-treatment titre.

Figure 2. Efficacy of telavancin (TLV; 40 mg/kg, sc, every 12 h), nafcillin (NAF; 40 mg/kg, sc, every 4 h), vancomycin (VAN; 110 mg/kg, sc every 12 h) and linezolid (LZD; 80 mg/kg, iv, every 12 h) in the murine MSSA pneumonia model (ATCC 29213).
reduction in MRSA inoculum size after 8 h. Similar findings were observed in the present study against MSSA in vitro. At concentrations up to 16 × MIC, the rate and magnitude of bactericidal activity with telavancin were comparable to those of nafcillin, but superior to those of vancomycin and linezolid. The reason for the modest in vivo efficacy of nafcillin, despite its rapid bactericidal activity in vitro, is unclear at present.

A caveat of this study is that only one strain of MSSA was studied. Therefore, these results may not be extrapolated to include other strains of MSSA. It should be noted, however, that telavancin has shown potent in vitro activity against many diverse and contemporary strains of this pathogen.

In summary, telavancin has potent and rapid bactericidal activity against MSSA in vitro and produces significantly greater reductions in lung titre in mice infected with MSSA versus nafcillin, vancomycin and linezolid. Therefore, these data support the clinical development of telavancin and infer that this compound is a promising candidate for the treatment of staphylococcal pneumonia.

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Transparency declarations

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