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Original Article

# Efficacy of tedizolid against methicillin-resistant *Staphylococcus aureus* and *Peptostreptococcus anaerobius* in thigh mixed-infection mouse model

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ABSTRACT

*Objective:* The purpose of this study is to compare the antimicrobial activity of human simulated exposures of tedizolid 200 mg daily, and linezolid 600 mg every 12 h for the treatment of complicated skin and skin structure infection (cSSSI) caused by MRSA and *Peptostreptococcus anaerobius* in both the neutropenic mice thigh mixed-infection models.
*Material and method:* Tedizolid phosphate and linezolid were used for all *in vivo* testing. A total of one MRSA and two *P. anaerobius* isolates were utilized. Antimicrobial efficacy was calculated for each isolate as the change in bacterial numbers ($\Delta\log_{10}$ CFU/ml) obtained in the treated mice after 24 h compared with the numbers in the starting control animals (0 h).
*Results:* The tedizolid and linezolid MICs for MRSA was 0.25 and 2 μg/ml. Tedizolid MIC for *P. anaerobius* was 0.12 μg/ml, and linezolid MICs for two *P. anaerobius* isolates were 0.5 and 1 μg/ml. In mixed infection model, tedizolid therapy showed similar antimicrobial activities for one MRSA and two *P. anaerobius* isolates evaluated, compared with linezolid therapy. Additionally, when comparing the activity of tedizolid and linezolid monotherapy between single infection and mixed infection model, antimicrobial activities of both antimicrobials were attenuated when mixed infection model was used.
*Conclusion:* In the neutropenic murine thigh infection model, human simulated exposures of tedizolid and linezolid resulted in similar efficacies against MRSA, even though single and mixed infection models were used. These data support the clinical utility of tedizolid for use against MRSA and *P. anaerobius* in the treatment of cSSSI.


## 1. Introduction

Since 1960, methicillin-resistance in *Staphylococcus aureus* has become a critical problem in healthcare and community settings [1–4]. The numbers of patients with methicillin-resistant *S. aureus* (MRSA) skin infections have increased in recent years, as have cases of complicated skin and soft tissue infection (cSSSI) [5]. As a result of this changing epidemiology and limited therapeutic armamentarium available to the clinician due to both the emergence of resistance and the potential for drug toxicities, new therapies targeted at this pathogen are needed.

Tedizolid is a novel oxazolidinone antimicrobial which has high bioavailability, penetration, and tissue distribution when administered orally or intravenously. Its once-daily dosing (200 mg daily) has shown favorable results in the treatment of acute bacterial skin and skin-structure infections in clinical trial, with potent activity against *S. aureus* isolates, including MRSA [6,7]. Moreover, no hematological adverse effects have been reported associated with tedizolid when used at the therapeutic dose of 200 mg in clinical

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trials of up to 3 weeks of tedizolid administration. Hence, tedizolid is an attractive candidate for clinical evaluation in cSSSI [8,9].

Necrotizing fasciitis (NF), is a type of cSSSI, is an uncommon subcutaneous tissue and superficial fascia infection that is associated with systemic toxicity, a fulminant course, and a mortality rate of 30–60% [10,11]. In clinical study, bacterial growth was noted in 81 of 83 (98%) of specimens from patients with NF. Then, aerobic bacteria only were recovered in 8 (10%) specimens, anaerobic bacteria only were recovered in 18 (22%) specimens, and mixed aerobic-anaerobic floras were recovered in 55 (68%) specimens. The predominant anaerobes were *Peptostreptococcus* spp., *Prevotella* and *Porphyromonas* spp., *Bacteroides fragilis* group, and *Clostridium* spp. These data highlight the polymicrobial nature of NF [10–12].

Therefore, to explore the best treatment of cSSSI, especially for NF, it is important to evaluate antimicrobial activity with mixed-infection model of aerobic-anaerobic floras. However, a few literatures have been available. The purpose of this study is to compare the antimicrobial activity of human simulated exposures of tedizolid 200 mg daily, and linezolid 600 mg every 12 h for the treatment of complicated skin and soft tissue infection (cSSSI) caused by MRSA and *Peptostreptococcus anaerobius* in the neutropenic mice thigh mixed-infection models.

## 2. Material and methods

### 2.1. Antimicrobial test agents

Tedizolid (TR-700, lot: 4130103, Bayer Pharma AG, Leverkusen, Germany) and linezolid (Sigma, Japan) was used for *in vitro* testing. And tedizolid phosphate (TR-701, lot: 4130103, Bayer Pharma AG, Leverkusen, Germany) and linezolid was used for all *in vivo* testing. Immediately prior to each *in vivo* experiment, each antimicrobial was weighed, reconstituted as pharmaceutical company recommended, and further diluted in appropriate diluents to achieve the desired concentration. Each solution was stored under refrigeration and discarded 24 h after reconstitution. For the animal studies, drugs were administered by the subcutaneous (SQ) routs in a solution prepared from the reconstituted powder as directed by the manufacturer.

### 2.2. Microbiology

A total of one MRSA and two *P. anaerobius* isolates with varying phenotypes (linezolid's antimicrobial susceptibility) were utilized. For each experiment, a bacterial strain was cultured overnight on blood agar plates to confirm the purity and viability of the microbe. Then, a few colonies were taken from the overnight agar culture and grown in Trypticase Soy Agar plates with 5% sheep blood (Becton, Dickinson & Co.; Sparks, MD) for MRSA and Burusera HK nutrient agar (Kyokuto, Tokyo, Japan) for *P. anaerobius*. The bacterial suspensions were diluted to the desired concentrations and used immediately. The bacterial densities in the suspensions were confirmed by quantitative cultures.

### 2.3. Susceptibility studies

The minimum inhibitory concentration (MIC) of tedizolid and linezolid was determined in triplicate for all test organisms by broth microdilution as described by CLSI guidelines [13] and the modal MIC was used to characterize the isolates. For *in vitro* each experiment, tedizolid was dissolved in dimethyl sulfoxide (DMSO) and then further diluted with medium to the desired concentrations. These drug solutions were used immediately.

### 2.4. Neutropenic thigh infection model

Specific-pathogen-free, female ICR mice weighing approximately 20–25 g was obtained from Charles River Laboratories Japan, Inc., (Yokohama, Japan) and utilized throughout the experiment. The animals were maintained and utilized in accordance with National Research Council recommendations and be provided food and water *ad libitum*. Mice was rendered transiently neutropenic by injecting cyclophosphamide intra peritoneal at a dose of 150 mg/kg of body weight at four days before inoculation and 100 mg/kg of body weight at one day before inoculation. Isolates to be inoculated into the thighs were previously frozen at −80 °C in skim milk. Two transfers of the organism was performed onto Trypticase Soy Agar plates with 5% sheep blood (Becton, Dickinson & Co.; Sparks, MD) and Burusera HK nutrient agar, and placed into an incubator at 37 °C for approximately 24 h. After an 18–24 h incubation of the 2nd transfer, a bacterial suspension of approximately $10^7$ CFU/ml was made for inoculation. Final inoculum concentrations were confirmed by serial dilution and plating techniques. Thigh infection with each of the test isolates will be produced by intramuscular injection of 0.1 ml of the inoculum into each thigh of the mice 2 h prior to the initiation of antimicrobial therapy.

### 2.5. Bacterial density studies

The purpose of this study was to assess the *in vivo* activity of human simulated tedizolid (200 mg daily) and linezolid (600 mg twice daily) against MRSA and *P. anaerobius* exhibiting various phenotypic. Tedizolid and linezolid treatments were begin 2 h initiation of infection. The following dosing regimens have been determined previously [14,15]. The regimen simulating tedizolid 200 mg daily given intraperitoneal at 0 h, and the regimen simulating linezolid 600 mg twice daily given subcutaneously at 0 and 12 h. Control animals received sterile normal saline in the same volume, route, and schedule as the active drug regimen. All animals were euthanized by $CO_2$ exposure followed by cervical dislocation by 24 h as appropriate. After sacrifice, the thighs were removed and individually homogenized in normal saline. Serial dilutions were plated on an appropriate agar media for CFU/ml determination.

### 2.6. Analyses

For the purposes of these studies, efficacy was calculated for each isolate as the change in bacterial numbers ($\Delta\log_{10}$ CFU/ml) obtained in the treated mice after 24 h compared with the numbers in the starting control animals (0 h). Efficacy amongst treatment regimens was compared using a Student *t* test of a Mann–Whitney *U* test if the data are not normally distributed. An analysis of variance was also be utilized to determine differences in $\Delta\log_{10}$ CFU/ml among the array of isolates. A *p* value of 0.05 was defined a priori as statistically significant.

## 3. Results

### 3.1. Bacterial isolates

One MRSA and two *P. anaerobius* isolates (19 and 18) were evaluated in this study. The tedizolid (TR-700: active form) and linezolid minimum inhibitory concentration (MIC) for MRSA was 0.25 and 2 μg/ml, respectively. Tedizolid MICs for both *P. anaerobius isolates* (19 and 18) were 0.12 μg/ml. And linezolid MICs for *P. anaerobius* isolates (19 and 18) were 0.5 and 1 μg/ml, respectively.