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Comparative Efficacies of Tedizolid Phosphate, Linezolid, and Vancomycin in a Murine Model of Subcutaneous Catheter-Related Biofilm Infection Due to Methicillin-Susceptible and -Resistant *Staphylococcus aureus*

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Tedizolid, a novel oxazolidinone, exhibits bacteriostatic activity through inhibition of protein synthesis. The efficacies of tedizolid, linezolid, and vancomycin were compared in a murine catheter-related biofilm infection caused by methicillin-susceptible and -resistant *Staphylococcus aureus* (MSSA and MRSA, respectively) strains engineered for bioluminescence. We observed significantly improved efficacy in terms of decreased *S. aureus* densities and bioluminescent signals in the tedizolid-treated group versus the linezolid- and vancomycin-treated groups in the model of infection caused by the MSSA and MRSA strains.

Staphylococcus aureus is a leading cause of skin and skin structure infections and is particularly associated with intravenous (i.v.) catheters (1–4). Despite the current use of newer antibiotics, infections due to *S. aureus* remain a significant problem. The emergence of methicillin-resistant *S. aureus* (MRSA) and high rates of vancomycin clinical failures emphasize this public health threat (5, 6). Therefore, potential alternative strategies for the treatment of such infections are urgently needed.

Tedizolid is a novel oxazolidinone derivative that has potent activity against staphylococci and enterococci (7, 8). It exerts bacteriostatic microbial activity through inhibition of protein synthesis by binding to the 50S ribosomal subunit of the bacteria. It has been reported that tedizolid is more active against staphylococci and enterococci than linezolid is *in vitro* (9, 10).

In this investigation, we evaluated (i) the MICs (11) and *in vitro* killing activities (12, 13) of tedizolid, linezolid, and vancomycin against *lux* mutant methicillin-susceptible *S. aureus* (MSSA) (Xen29) and MRSA (Xen30) strains (4, 14, 15), (ii) the influence of these antibiotics on biofilm formation (16, 17), and (iii) the real-time *in vivo* efficacy of tedizolid versus that of linezolid and vancomycin in a well-characterized model of murine subcutaneous catheter-related infection (18) caused by the MSSA or MRSA strain by using a novel bioluminescence *in vivo* imaging system (IVIS).

The IVIS was developed to provide a sensitive and noninvasive technique for rapid and real-time monitoring of therapeutic efficacy (4, 14, 19–21). In the murine subcutaneous catheter-related infection model, BALB/c mice (female, 18 to 22 g; Jackson Laboratory) were infected by implanting a precolonized Teflon catheter segment (1 cm) inoculated with the bioluminescent strains at 1×10^6 CFU/catheter. At 3 days after catheter implantation, animals were randomized to receive (i) control treatment with the vehicle, (ii) tedizolid phosphate at 10 mg/kg i.v. twice a day (bid), (iii) linezolid at 80 mg/kg i.v. bid, or (iv) vancomycin at 110 mg/kg subcutaneously (s.c.) bid. These antibiotic doses were chosen to simulate pharmacokinetic values similar to those achieved by the recommended dosing of humans, i.e., 200 mg of tedizolid i.v. once daily (22), 600 mg of linezolid i.v. bid (23), and 1 g of vancomycin i.v. bid (24). Treatments lasted 3 and 6 days for MSSA and MRSA infections, respectively. At 24 h after the last antibiotic dose, half of the untreated and tedizolid-treated animals were sacrificed for evaluation of antibiotic efficacy. The other half of the survivors were left untreated for an additional 3 days for assessment of relapse. At sacrifice, catheters were quantitatively cultured by using standard assays of CFU counts per catheter (25). In addition, animals were serially imaged daily after infected-catheter implantation for bioluminescent signals (BLS) with the IVIS (Caliper Life Sciences). BLS were expressed by using a pseudocolor scale, with red representing the most intense luminescence and blue representing the least intense luminescence (21).

The MICs of tedizolid, linezolid, and vancomycin for study strains Xen29 and Xen30 were 0.25 and 0.25, 1.0 and 2.0, and 1.0 and 1.0 μg/ml, respectively. Tedizolid prevented substantial regrowth between 6 and 24 h of incubation (Fig. 1). However, linezolid could not prevent the regrowth of both MSSA and MRSA strains at the MIC or 2× MIC. Vancomycin exhibited time-dependent bactericidal activity against both study strains. No significant differences in the *in vitro* activities of tedizolid, linezolid, and vancomycin were observed between the study MSSA and MRSA strains (Fig. 1).

The two study MSSA and MRSA strains formed good biofilm (range of optical densities at 490 nm, 1.7 to 2.1) (Fig. 2). Interestingly, sublethal levels (0.5× MIC) of tedizolid, linezolid, or vancomycin did not induce biofilm formation in either study strain. Of note, linezolid is more effective than tedizolid or vancomycin against MSSA biofilm formation, and the ozaxolidinones are

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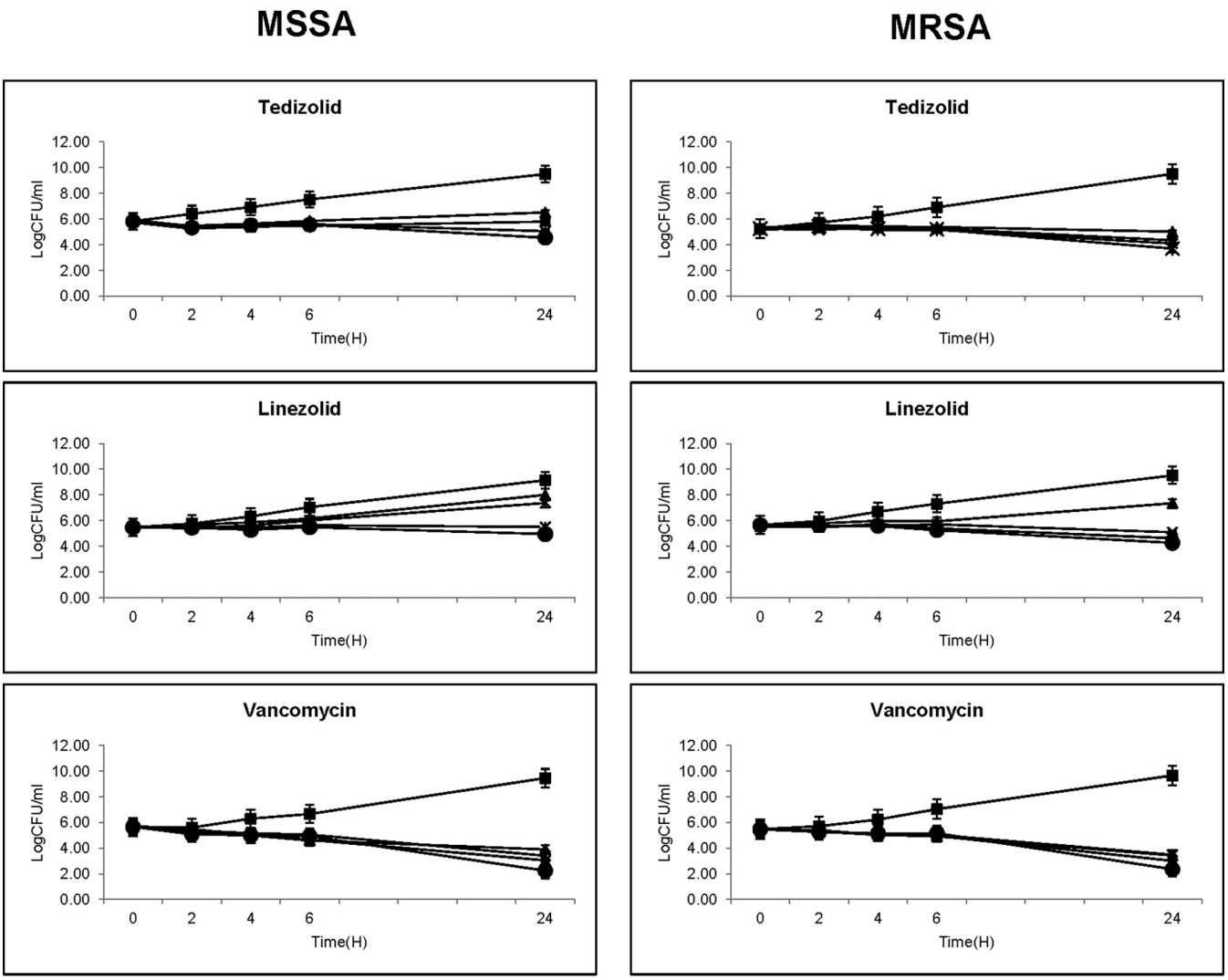

FIG 1 Tedizolid, linezolid, and vancomycin *in vitro* MSSA (Xen29) and MRSA (Xen30) time-kill curves. Symbols: ■, control; ▲, 1× MIC; X, 2× MIC; *, 5× MIC; ●, 10× MIC.

more active than vancomycin against MRSA strain biofilm formation. Importantly, all three antibiotics at the MIC to 10× MIC significantly reduced biofilm formation in both MSSA and MRSA strains.

The burden of organisms in catheters in the untreated controls, different therapies, and relapse groups in the murine model are shown in Tables 1 and 2. At the end of treatment, tedizolid phosphate therapy (10 mg/kg i.v. bid) produced significantly lower densities of both MSSA and MRSA bacteria in catheters than those found in untreated controls or vancomycin- and linezolid-treated animals. In addition, vancomycin and linezolid had no therapeutic efficacy in reducing MSSA densities, while linezolid showed a significant reduction of MRSA densities versus untreated controls in the model. Of importance, tedizolid treatment had no significant relapse after discontinuation of therapy. The BLS from animals treated with tedizolid showed a progressive reduction compared to that from linezolid- and vancomycin-treated groups (Fig. 3).

Our *in vitro* results demonstrated that the study MSSA and MRSA strains were susceptible to tedizolid (MICs of 0.25 μg/ml), linezolid (MICs of ≤2.0 μg/ml), and vancomycin (MICs of 1.0 μg/ml). In addition to these MIC data, *in vitro* time-kill studies showed that tedizolid had better anti-*S. aureus* activity than linezolid in that it prevented regrowth at 24 h of incubation. The *in vitro* findings in this study mirror those in previously published investigations of tedizolid and linezolid activity against *S. aureus* (9, 26). In the present study, we further evaluated the impact of tedizolid versus that of linezolid and vancomycin on biofilm formation in *S. aureus* and found that exposure of these antibiotics at the MIC to 10× MIC significantly decreased *S. aureus* biofilm formation. These findings differ from those of a previous study that showed that tedizolid had no activity when staphylococcal organisms were in a biofilm state (26).

Importantly, the present study translated the above-described *in vitro* outcomes into a clinically relevant catheter-related biofilm infection model in mice. Our results demonstrated that tedizolid had significantly better efficacy than linezolid and vancomycin treatments in reducing MSSA and MRSA densities in the murine

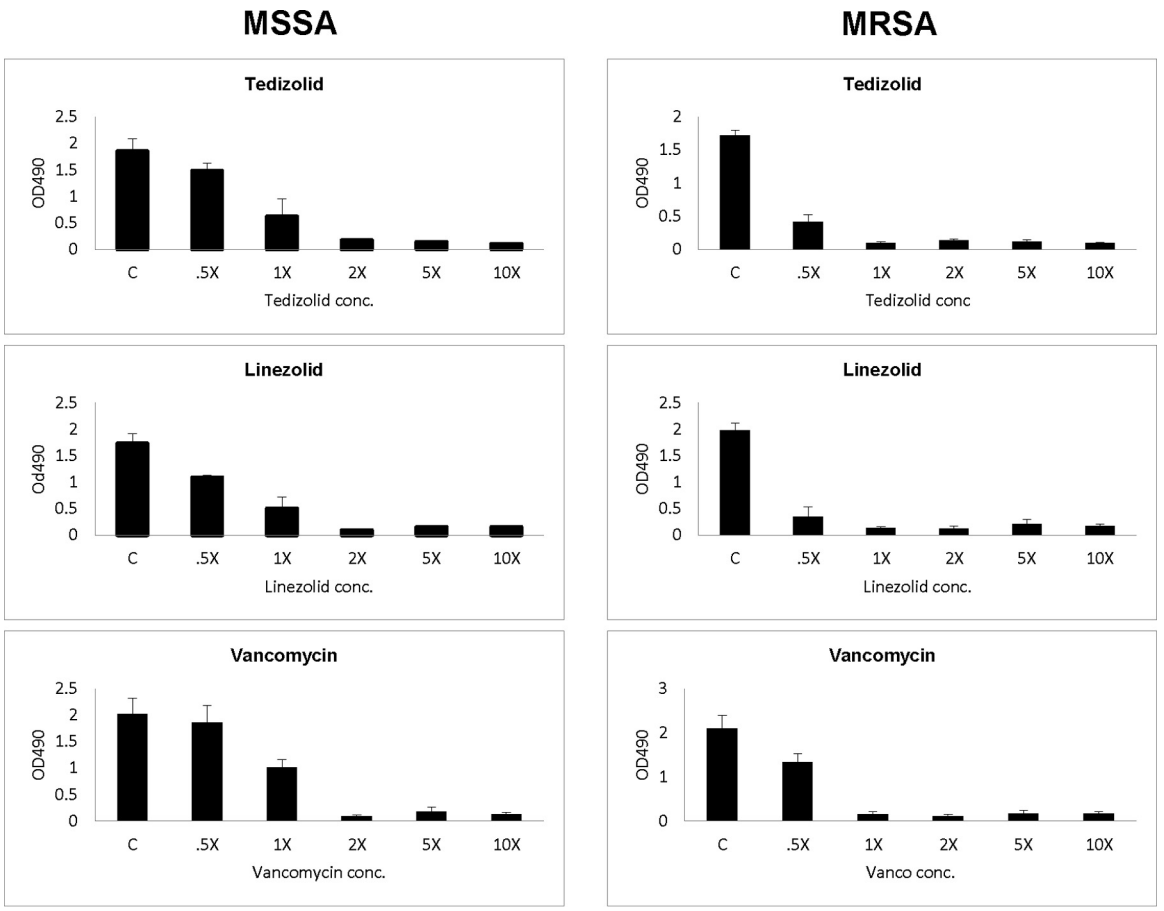

FIG 2 Impact of tedizolid, linezolid, or vancomycin (from 0.5× MIC to 10× MIC) on MSSA (Xen29) and MRSA (Xen30) biofilm formation. OD490, optical density at 490 nm; C, control.

subcutaneous catheter-related biofilm infection model. In addition, it is notable that tedizolid had significant efficacy in the experimental model of MSSA infection, even with short-course (3 days) therapy, and there was no substantial relapse after 3 days without treatment. On the other hand, neither linezolid nor vancomycin was efficacious in producing lower MSSA densities than those in the control group in the model. These results suggest that tedizolid had significantly better efficacy in decreasing *S. aureus* densities in this model of catheter-related biofilm infection caused by MSSA and MRSA strains.

TABLE 1 Efficacies of tedizolid, vancomycin, and linezolid in a murine subcutaneous catheter-related infection due to MSSA strain Xen29

Regimen (no. of samples)	Mean $\log_{10}$ no. of CFU/catheter ± SD	*P* value(s)
Treatement[a]		
Control (20)	7.50 ± 0.33	
Tedizolid phosphate, 10 mg/kg i.v. bid (22)	5.49 ± 0.62	<1.37E−07 vs control, <2.74E−07 vs linezolid, <3.61E−09 vs vancomycin
Linezolid, 80 mg/kg i.v. bid (20)	7.41 ± 0.48	<0.56 vs control
Vancomycin, 110 mg/kg s.c. bid (20)	7.46 ± 0.27	<0.79 vs control
Relapse		
Control (20)	7.13 ± 0.42	
Tedizolid phosphate, 10 mg/kg i.v. bid (20)	5.77 ± 0.66	<6.88E−09 vs control relapse

[a] Treatment lasted 3 days.

TABLE 2 Efficacies of tedizolid, vancomycin, and linezolid in a murine subcutaneous catheter-related infection due to MRSA strain Xen30

Regimen (no. of samples)	Mean $\log_{10}$ no. of CFU/catheter ± SD	*P* value(s)
Treatment[a]		
Control (20)	7.14 ± 0.37	
Tedizolid phosphate, 10 mg/kg i.v. bid (20)	5.70 ± 0.53	<3.07E−06 vs control, <0.03 vs linezolid, <0.002 vs vancomycin
Linezolid, 80 mg/kg i.v. bid (20)	6.47 ± 0.57	<0.05 vs control
Vancomycin, 110 mg/kg s.c. bid (20)	6.64 ± 0.83	<0.07 vs control
Relapse		
Control (20)	6.63 ± 0.53	
Tedizolid phosphate, 10 mg/kg i.v. bid (20)	5.34 ± 0.73	<0.0002 vs control relapse

[a] Treatment lasted 6 days.

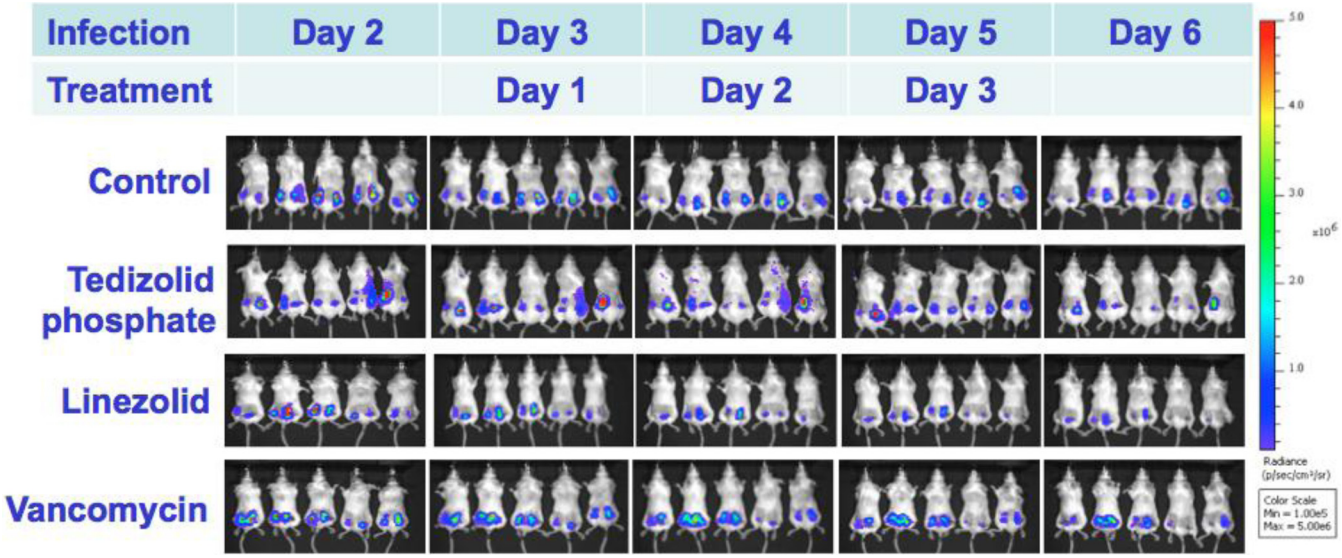

FIG 3 Real-time monitoring of the efficacy of tedizolid, linezolid, and vancomycin in a murine subcutaneous catheter-related MSSA biofilm model.

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