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EXPERIMENTAL THERAPEUTICS

Telavancin Is Active against Experimental Aortic Valve Endocarditis Caused by Daptomycin- and Methicillin-Resistant *Staphylococcus aureus* Strains

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ABSTRACT We compared the efficacy of telavancin (TLV) and daptomycin (DAP) in an experimental rabbit endocarditis model caused by two clinically derived daptomycin-resistant (DAP^r) methicillin-resistant *Staphylococcus aureus* (MRSA) strains. TLV treatment significantly reduced MRSA densities in all target tissues and increased the percentage of these organs rendered culture negative compared to those with the untreated control or DAP-treated animals. These results demonstrate that TLV has potent *in vivo* efficacy against DAP^r MRSA isolates in this invasive endovascular infection model.

KEYWORDS TLV, DAP^r MRSA, endocarditis, daptomycin resistance, MRSA, telavancin, infective endocarditis

Staphylococcus aureus is the most common cause of endovascular infections, including infective endocarditis (IE) (1). Despite the use of new antibiotics, such as daptomycin (DAP), the morbidity and mortality associated with *S. aureus* infections remain unacceptably high (2). DAP is a lipopeptide antibiotic with activity against a wide range of Gram-positive bacteria, including methicillin-resistant *S. aureus* (MRSA) (3). However, there have been increasing reports in which initially DAP-susceptible (DAP^s) MRSA strains have developed DAP resistance (DAP^r) both *in vitro* and *in vivo* as a result of exposure to DAP (4, 5).

Telavancin (TLV) is a lipoglycopeptide agent with a dual mechanism of action: cell wall synthesis inhibition and depolarization of the bacterial cell membrane (6). Recently, we studied five clinically derived DAP^s/DAP^r MRSA isogenic strain pairs isolated from patients who failed DAP therapy for TLV *in vitro* susceptibility (7). We found that all five MRSA strain sets were susceptible to TLV (MICs, ≤0.38 μg/ml), using the original MIC testing methods (8). Importantly, TLV therapy produced a significant MRSA density reduction in target tissues versus untreated or DAP-treated animals in experimental IE due to a single DAP^r strain, REF2145 (7). These results demonstrated that TLV has potent bactericidal activity both *in vitro* and *in vivo* against DAP^r MRSA strains (7). However, only one DAP^r MRSA strain was tested in the IE model (7). In addition, the Clinical and Laboratory Standards Institute revised the antimicrobial susceptibility testing method for TLV in 2014 (9). The revised method provides more precise and reproducible TLV MICs and demonstrates that the previous technique underestimated the *in vitro* TLV potency (9, 10). Moreover, the revised CLSI methods decreased the TLV MIC interpretive breakpoint criterion for susceptibility for *S. aureus* from ≤1.0 μg/ml to ≤0.12 μg/ml (9). Therefore, in the current studies, we (i) retested the TLV MICs of the five clinically derived DAP^s/DAP^r MRSA strain pairs by using the revised CLSI method

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TABLE 1 Susceptibilities of TLV and DAP on DAP^s/DAP^r MRSA strain pairs

Strain	Clinical source of isolated DAP^s/DAP^r strain pairs	MIC (μg/ml)[a]	
		TLV (O/R)	DAP
$A_{0.5}$	Bloodstream	0.25/0.06	0.5
$B_{2.0}$[b]	Bloodstream	0.38/0.12	2.0
SA675	Endocarditis	0.38/0.06	0.25
SA684[b]	Endocarditis	0.38/0.12	2.0
MRSA11/11	Endocarditis	0.25/0.06	0.38
REF2145	Endocarditis	0.38/0.12	4.0
A214	Bloodstream	0.25/0.06	0.5
A215	Bloodstream	0.38/0.12	3.0
L282	Bloodstream	0.25/0.06	0.38
L283	Bloodstream	0.38/0.12	2.0

[a]O, original method (8); R, revised method (20).
[b]DAP^r strains used in the *in vitro* time-kill assays and *in vivo* IE model in the current study.

(10), (ii) performed *in vitro* time-kill assays based on the new TLV MICs, and (iii) investigated the therapeutic efficacy of TLV versus DAP in the IE model due to two additional clinically derived DAP^r MRSA strains ($B_{2.0}$ and SA684).

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Using the revised broth microdilution method, which includes dimethyl sulfoxide (DMSO) for panel production and polysorbate 80 (P-80), we found that TLV MICs were 3- to 6-fold lower than those in the previous results (Table 1) (7). These results were consistent with recent reports indicating that the previous method underestimated the *in vitro* TLV potency (10).

The *in vitro* time-kill curves were performed based on the new TLV MICs observed in this study, with an initial inoculum of 10^5 or 10^7 CFU/ml of study strain (to encompass bacterial counts commonly achieved in target tissues of animals with experimental IE) (12–14). For these assays, we prioritized two DAP^r MRSA strains ($B_{2.0}$ and SA684), initially isolated from patients who experienced DAP therapy failure (15, 16). At 10^5 CFU/ml initial inoculum, TLV at 2 times the MIC and 5 times the MIC prevented regrowth of both DAP^r MRSA strains (Fig. 1A and B), but DAP at only 5 times the MIC prevented regrowth of one DAP^r MRSA strain (Fig. 1F). At 10^7 CFU/ml initial inoculum, only TLV at 5 times the MIC (Fig. 1C and D), but no DAP concentration (Fig. 1G and H), was effective at preventing the regrowth of both DAP^r MRSA strains. These *in vitro* time-kill analyses revealed that TLV was more active than DAP against the two DAP^r MRSA strains.

For the *in vivo* experiments, we demonstrated that the infective dose required to infect 95% of animals (ID_{95}) of our two DAP MRSA strains in the IE model was 10^4 CFU/animal (data not shown). For the efficacy studies, animals were infected with this ID_{95} inoculum. At 24 h after infection, animals were randomly assigned to one of the four groups (10 animals/group): (i) untreated controls; (ii) TLV at 30 mg/kg of body weight, intravenously (i.v.), twice daily, which simulates the pharmacokinetic (PK) profile of the recommended human clinical dose (10 mg/kg, i.v. once daily) (7, 17, 18); (iii) DAP at 12 mg/kg, i.v. once daily, which mimics the human-like PK of the human standard dose (6 mg/kg, i.v., once daily) (15, 18); or (iv) DAP at 18 mg/kg, i.v. once daily, which mimics the human-like PK of high-dose DAP (10 mg/kg, i.v., once daily) (15). Treatment lasted for 3 days. At 24 h after the last therapeutic doses, antibiotic-treated animals were sacrificed. Control animals were euthanized at 24 h postinfection to determine the bacterial density in target tissue before antibiotic therapy. At sacrifice, the target tissues were removed and quantitatively cultured. Target tissue counts were expressed as mean $\log_{10}$ CFU/g of tissue $\pm$ standard deviation (SD). To compare tissue MRSA counts among the regimens, a nonparametric Kruskal-Wallis test was used. *P* values of <0.05 were considered statistically significant.

We observed that only TLV treatment significantly reduced MRSA densities in all three target tissues in the IE model due to two DAP^r MRSA strains versus untreated controls and DAP-treated groups (Table 2). Importantly, TLV-treated rabbits had 71 to

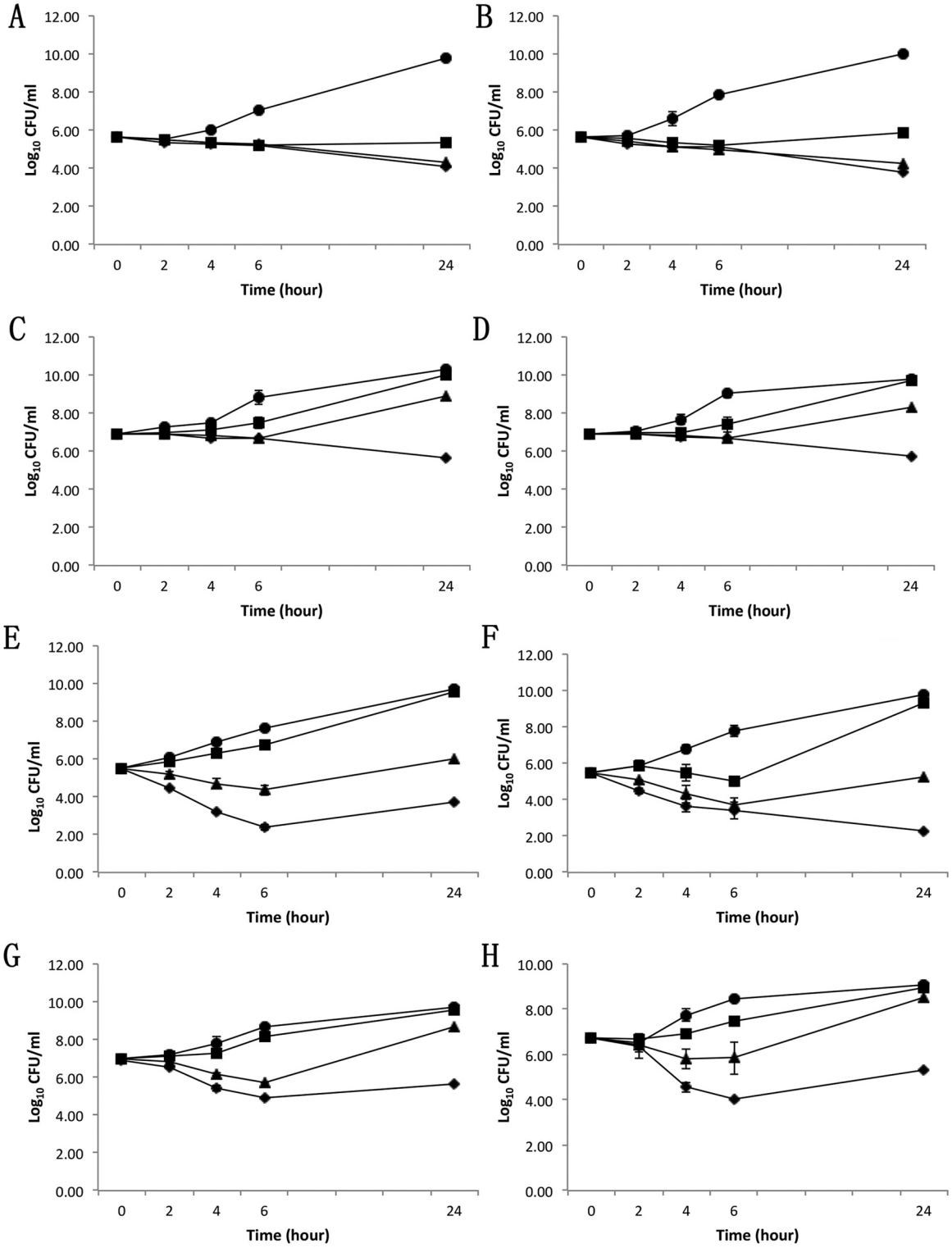

FIG 1 *In vitro* TLV and DAP time-kill curves against DAP^r MRSA strains $B_{2.0}$ (panels A and C for TLV, and panels E and G for DAP) and SA684 (panels B and D for TLV, and panels F and H for DAP) at initial inocula of 10^5 CFU/ml and 10^7 CFU/ml. Time-kill experiments were performed using Mueller-Hinton broth in the presence of 0 (●), 1× (■), 2× (▲), and 5× (◆) the MICs of TLV.

100% culture-negative target tissues (71% and 100% in animals infected with the $B_{2.0}$ and SA684 strains, respectively), while DAP therapy did not sterilize any tissue cultures (data not shown). In addition, 29% mortality was observed in the DAP 12 mg/kg treatment group and 0% mortality in the TLV treatment groups (data not shown).

TABLE 2 MRSA density in target tissue in IE model caused by DAP^r MRSA strain $B_{2.0}$ or SA684

Treatment group (no. of animals)[a]	Log_{10} CFU/g of tissue (mean ± SD)		
	Vegetation	Kidney	Spleen
Strain $B_{2.0}$			
Control (9)	8.17 ± 0.41	6.32 ± 0.86	6.13 ± 0.72
DAP, 12, once daily (7)	8.39 ± 0.67	6.38 ± 0.80	5.86 ± 0.60
DAP, 18, once daily (8)	7.54 ± 0.94	4.10 ± 0.50[b]	4.35 ± 0.79[b]
TLV, 30, b.i.d. (8)	0.66 ± 0.11[c]	0.49 ± 0.16[c]	0.53 ± 0.25[c]
Strain SA684			
Control (8)	8.57 ± 0.34	6.94 ± 0.77	6.40 ± 0.85
DAP, 12, once daily (7)	8.72 ± 0.55	7.01 ± 0.65	6.66 ± 0.77
DAP, 18, once daily (7)	8.49 ± 0.47	6.91 ± 0.80	5.81 ± 0.68
TLV, 30, b.i.d. (7)	1.31 ± 0.96[b]	0.84 ± 0.66[b]	1.00 ± 0.76[b]

[a]All drug dosages are given in milligrams per kilogram of body weight, and all drugs were administered intravenously. b.i.d., twice daily.
[b]$P < 0.005$ versus untreated controls.
[c]$P < 0.001$ versus untreated controls and DAP-treated groups.

These results were consistent with our prior single-strain investigation of TLV's excellent activity in experimental IE due to DAP^r MRSA strains (7). It may not be suitable to directly compare TLV versus DAP efficacy in the IE model due to DAP^r MRSA strains because of their inherent reduced susceptibility to DAP. However, it is important in demonstrating that TLV does have great activity against infections caused by these strains, while the use of DAP would not be an appropriate option. In addition to DAP^r MRSA strains, other studies also demonstrated that TLV had significantly better efficacy than with vancomycin and DAP in the IE models due to a broad range of MRSA strains, including vancomycin-intermediate *S. aureus* (VISA) and glycopeptide-intermediate *S. aureus* (GISA) (17–19). Taken together, these results suggest that TLV may be a viable alternative for the treatment of IE caused by MRSA strains resistant to other glycopeptide or lipopeptide antibiotics.

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