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Telavancin (TLV) Is More Bactericidal Than Vancomycin (VAN) against Both Extracellular and Intracellular (THP-1 Macrophages) *Staphylococcus aureus* with Different Resistance Phenotypes

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ABSTRACT

Background: TLV is a novel lipoglycopeptide (presently in late clinical trial evaluation) with marked bactericidal activity against *S. aureus*, including strains resistant to conventional glycopeptides. We compare here TLV and VAN activity against extracellular and intracellular *S. aureus* with different resistance phenotypes.

Methods: MICs were determined by microdilution in Mueller-Hinton broth (for methicillin-susceptible *S. aureus* [MSSA]) or in Mueller-Hinton broth supplemented with 2% sodium chloride (for methicillin-resistant *S. aureus* [MRSA]). Bacteria in cell culture medium (extracellular) or in THP-1 infected macrophages (intracellular; see Barcia-Macay et al. *Antimicrob Agents Chemother*. 2006;50:841-851) were exposed for up to 24 hours to VAN or TLV at increasing extracellular concentrations.

Results: The table shows the MICs of VAN and TLV, together with the changes in cfu from the initial inoculum measured extracellularly and in THP-1 microphages at a concentration equal to the human C_{max} (VAN: 50 mg/L; TLV: 90 mg/L [total drug]). Extracellularly, TLV displayed a larger bactericidal effect than VAN, reaching the limit of detection within 6 hours for MSSA and MRSA. Intracellularly, VAN was almost static, while TLV showed a bactericidal effect, which, however, was less pronounced for vancomycin-resistant *S. aureus* (VRSA) compared with other strains.

Strain (phenotype)	MIC, mg/L		Difference in log cfu from Time 0			
			Extracellular (6 h)		Intracellular (24 h)	
	VAN	TLV	VAN	TLV	VAN	TLV
ATCC 25923 (MSSA)	1	0.5	-2.9 ± 0.1	-5*	-0.6 ± 0.1	-2.1 ± 0.1
ATCC 33591 (MRSA)	2	0.5	-3.8 ± 0.2	-5*	-0.6 ± 0.1	-1.9 ± 0.1
NRS23 (VISA)	4	0.5	-1.0 ± 0.1	-3.0 ± 0.1	-0.2 ± 0.2	-2.1 ± 0.1
VRS1 (VRSA)	256	4	NA	-2.5 ± 0.1	NA	-1.1 ± 0.1

*The cfu counts were below the limit of detection (>5 log decrease; NA).

Conclusions: TLV proved more effective in this model than VAN against both extracellular and intracellular *S. aureus*, irrespective of resistance phenotype.

INTRODUCTION

Selecting an appropriate treatment for *S. aureus* infections currently involves 2 major issues:

1. Increasing resistance to the available antimicrobial agents for strains of MRSA, vancomycin-intermediate *S. aureus* (VISA), and VRSA narrows the choice of antibiotics.
2. The capacity of *S. aureus* to survive and multiply within eukaryotic cells protects it from the bactericidal action of many antibiotics and is probably associated with the persistent or recurrent character of infections (Lowy. *Trends Microbiol*. 2000;8:341-343).

TLV is a rapidly bactericidal semisynthetic glycopeptide, which, in contrast to VAN:

- maintains its activity against VISA and VRSA strains, owing to its multiple modes of action (Higgins et al. *Antimicrob Agents Chemother*. 2005;49:1127-1134).
- accumulates in macrophages (Gotfried et al. ICAAC 2005. Abstract A-14; Barcia-Macay et al. ICAAC 2005. Abstract A-1831).

The aim of this study was to compare the extracellular and intracellular activity of VAN and TLV toward staphylococci harboring different resistance phenotypes.

METHODS

1. EXTRACELLULAR ACTIVITY

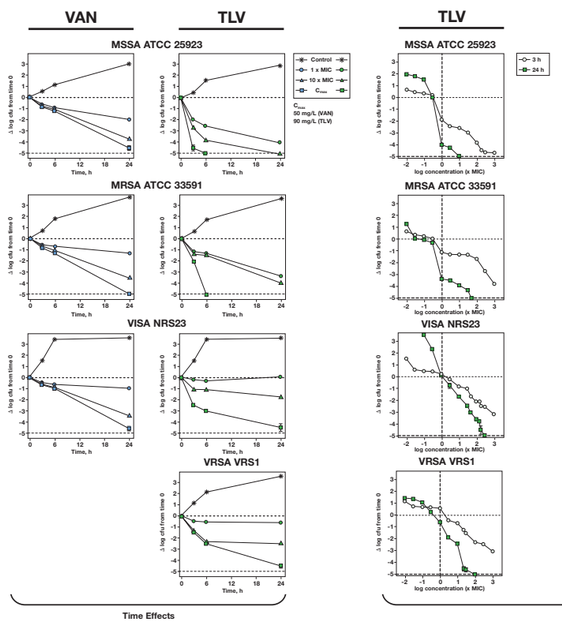
MICs were determined by microdilution in Mueller-Hinton broth supplemented by 2% sodium chloride for MRSA. Extracellular activity was evaluated by performing killing curve experiments in Roswell Park Memorial Institute medium supplemented with 10% fetal calf serum (Barcia-Macay et al. *Antimicrob Agents Chemother.* 2006;50:841-851).

2. INTRACELLULAR ACTIVITY

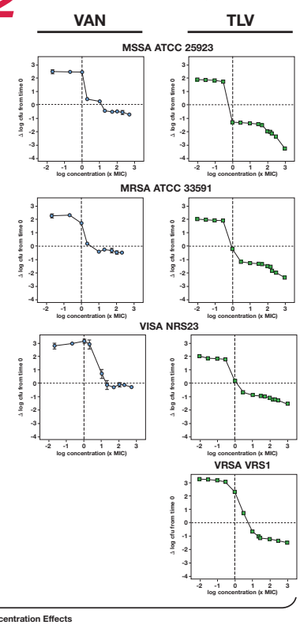
Intracellular infection was produced by a 1-hour incubation with serum-opsonized bacteria (4 bacteria/THP-1 macrophages), washing with gentamicin for MSSA, MRSA, and VISA or linezolid for VRSA (resistant to gentamicin) at 100 x MIC, and reincubation in fresh medium containing the tested antibiotic. The cfu/mg of cell protein was determined by plating cell lysates (Barcia-Macay et al. *Antimicrob Agents Chemother*. 2006;50:841-851).

RESULTS

1 Extracellular Activity



2 Intracellular Activity



3 MICs

Phenotype	Strain	Origin	MIC, mg/L	
			VAN	TLV
MSSA	25923	ATCC	1	0.5
MRSA	33591	ATCC	2	0.5
VISA	NRS23	NARSA	4	0.5
VRSA	VRS1	NARSA	>128	4

1. EXTRACELLULAR ACTIVITY

1.1 Time effects

VAN acted slowly, with a marked influence of concentration at 24 hours only. TLV activity was concentration-dependent at all times as well as faster than VAN activity, especially toward MSSA and MRSA.

1.2 Concentration effects

At 3 hours a bimodal response with respect to the concentration of TLV was clearly seen for both MSSA and MRSA. In contrast, there was a linear relationship with VISA and VRSA. At 24 hours the limit of detection was reached at concentrations ranging between ~10 and 100 x MIC.

2. INTRACELLULAR ACTIVITY

2.1 Concentration effects

VAN was only static even at high x MIC. TLV showed concentration-dependent bactericidal effects, with bimodal responses maintained for MSSA and MRSA.

CONCLUSIONS

1. TLV proved bactericidal toward both the extracellular and intracellular forms of *S. aureus*, irrespective of resistance phenotype.
2. Bimodality of dose response toward MSSA and MRSA may be related to the multiple modes of action of TLV as described by Higgins and colleagues (*Antimicrob Agents Chemother*. 2005;49:1127-1134).
3. VAN was bacteriostatic toward the intracellular form of *S. aureus* and slowly bactericidal toward the extracellular form.

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