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Antistaphylococcal Activity of TD-1792, a Multivalent Glycopeptide-Cephalosporin Antibiotic

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TD-1792 is a new multivalent glycopeptide-cephalosporin antibiotic with potent activity against Gram-positive bacteria. The *in vitro* activity of TD-1792 was tested against 527 *Staphylococcus aureus* isolates, including multidrug-resistant isolates. TD-1792 was highly active against methicillin-susceptible *S. aureus* (MIC_{90}, 0.015 μg/ml), methicillin-resistant *S. aureus*, and heterogeneous vancomycin-intermediate *S. aureus* (MIC_{90}, 0.03 μg/ml). Time-kill studies demonstrated the potent bactericidal activity of TD-1792 at concentrations of ≤0.12 μg/ml. A postantibiotic effect of >2 h was observed after exposure to TD-1792.

The global emergence of Gram-positive pathogens with decreased susceptibility to available therapies has become a major public health problem, with *Staphylococcus aureus* being of particular concern (2, 6). We initiated a program to design multivalent antibiotics optimized for activity against multidrug-resistant Gram-positive bacteria. The chemical design included covalent attachment of vancomycin through a chemically stable linker to a cephalosporin. The details concerning the discovery of the heterodimer TD-1792 (Fig. 1) have been described elsewhere (12). This agent exerts bactericidal activity against clinically relevant Gram-positive pathogens, including multidrug-resistant organisms, such as methicillin-resistant *S. aureus* (MRSA) and vancomycin-intermediate *S. aureus* (VISA) (10). In a randomized, double-blind phase 2 study of patients with complicated skin and skin structure infections (cSSSI), TD-1792 was found to be safe and noninferior to vancomycin with respect to efficacy (14).

A total of 527 clinical isolates of *S. aureus* collected worldwide at various hospitals from 2005 to 2007 were used in the MIC studies described here. Six strains of MSSA representing four distinct staphylococcal β-lactamases (types A, B, C, and D) were also studied (9). TD-1792 and THRX-169797 (representing the cephalosporin moiety of TD-1792) were prepared by Theravance, Inc. (South San Francisco, CA). All comparator antibiotics for MIC testing were provided by Trek Diagnostics (Cleveland, OH). Comparator antimicrobial agents included linezolid (Zyvox; Pfizer), nafcillin, penicillin G, and vancomycin (Sigma Chemical Co., St. Louis, MO). Susceptibility testing was performed using a broth microdilution assay following the recommended CLSI methodology (3).

The MIC results for TD-1792 and comparator agents are summarized in Table 1. On the basis of MIC_{90}, TD-1792 was the most active agent tested against clinical strains of MSSA (MIC_{90}, 0.015 μg/ml). TD-1792 was also found to be very active against a large group of MRSA isolates. The highest MIC of TD-1792 among all MRSA strains surveyed was 0.03 μg/ml. Based upon MIC_{90} comparisons, TD-1792 was 16-fold more active than daptomycin, 32-fold more active than vancomycin, and 128-fold more active than linezolid. A single daptomycin-nonsusceptible strain (MIC, 2 μg/ml) was identified. This isolate was susceptible to vancomycin (MIC, 1 μg/ml); the TD-1792 MIC for this isolate was 0.015 μg/ml.

All tested MRSA isolates underwent pulsed-field gel electrophoresis (PFGE) genotyping as described previously by Bae and colleagues (1). Among the 324 MRSA isolates evaluated, 208 (64.2%) were characterized by the USA typing schema. Of these 208 isolates, 181 (87.0%) were USA300 (TD-1792 MIC range, 0.008 to 0.03 μg/ml), and 20 (9.6%) were USA100 (TD-1792 MIC range, 0.008 to 0.03 μg/ml). Other phenotypes identified were as follows: USA 200 (1 isolate; TD-1792 MIC, 0.015 μg/ml), USA 400 (3 isolates; TD-1792 MIC range, 0.015 to 0.03 μg/ml), USA 500 (2 isolates; TD-1792 MICs, 0.015 μg/ml), and USA 600 (1 isolate; TD-1792 MIC, 0.015 μg/ml).

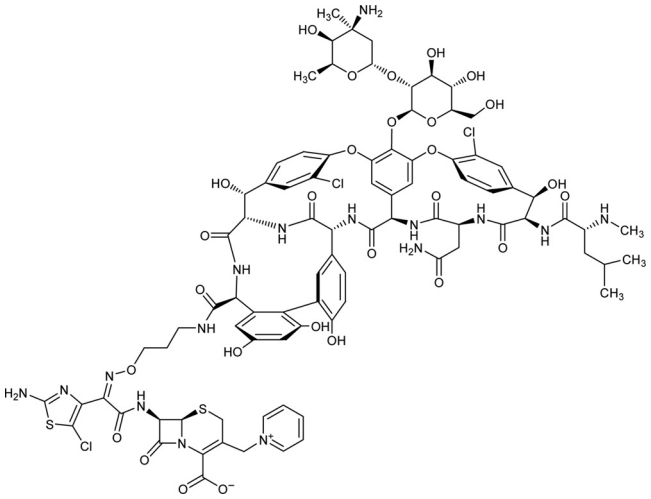

FIG 1 Chemical structure of TD-1792.

A collection of 39 *S. aureus* isolates confirmed as heterogeneous VISA (hVISA) by population analysis profiling (11), was also tested. For these isolates, the vancomycin MIC_{90} was 2 μg/ml.

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TABLE 1 *In vitro* activity of TD-1792 against *S. aureus* isolates

S. aureus (no. tested) and antibiotic	MIC (μg/ml)			% Susceptible[a]
	Range	50%	90%	
Methicillin susceptible (164)				
TD-1792	0.002–0.03	0.008	0.015	NA
Oxacillin	≤0.06–2	0.25	0.5	100
Vancomycin	≤0.25–2	1	1	100
Daptomycin	0.06–1	0.5	0.5	100
Linezolid	1–4	2	4	100
Clindamycin	≤0.5–>4	≤0.5	≤0.5	93.3
Ciprofloxacin	0.12–>8	0.5	2	89.0
Gentamicin	≤0.06–>16	0.5	2	97.0
Erythromycin	≤0.12–>16	1	>16	23.2
Trimethoprim-sulfamethoxazole	≤0.5/9.5–2/38	≤0.5/9.5	≤0.5/9.5	100
Methicillin resistant (324)				
TD-1792	0.008–0.03	0.015	0.03	NA
Vancomycin	≤0.25–2	1	1	100
Daptomycin	0.12–2	0.5	0.5	99.7
Linezolid	1–4	2	4	100
Clindamycin	≤0.5–>4	≤0.5	>4	66.4
Ciprofloxacin	0.12–>8	8	>8	26.2
Gentamicin	≤0.06–>16	1	>16	73.5
Erythromycin	0.5–>16	>16	>16	1.5
Trimethoprim-sulfamethoxazole	≤0.5/9.5–>4/76	≤0.5/9.5	≤0.5/9.5	90.7
Heterogeneous vancomycin intermediate (39)				
TD-1792	0.015–0.06	0.03	0.03	NA
Vancomycin	0.5–2	1	2	100
Daptomycin	0.25–2	0.5	1	97.4
Linezolid	1–2	2	2	100
Clindamycin	≤0.5–>4	>4	>4	5.1
Ciprofloxacin	2–>8	>8	>8	0
Gentamicin	0.5–>16	>16	>16	20.5
Erythromycin	>16	>16	>16	0
Trimethoprim-sulfamethoxazole	≤0.5/9.5–>4/76	>4/76	>4/76	48.7

[a] Susceptibility of each agent as defined by CLSI document M100-S21 (4).

TD-1792 demonstrated potent *in vitro* activity against this collection, with all MIC values being ≤0.06 μg/ml. One of the hVISA isolates was also nonsusceptible to daptomycin (MIC, 2 μg/ml); TD-1792 maintained an MIC value of 0.03 μg/ml against this strain.

Time-kill experiments were performed according to CLSI-defined methodology (13). TD-1792 demonstrated potent bactericidal activity at concentrations equal to two times the MIC (2× MIC) against all six *S. aureus* isolates tested (Table 2). Against both MSSA isolates tested, TD-1792 at 2× MIC resulted in a ≥3-$\log_{10}$ reduction by 4 h. Vancomycin, nafcillin, and cefazolin were also bactericidal but only by 24 h when tested at 8× their respective MICs. Against the three MRSA isolates tested, TD-1792 was bactericidal at all MIC multiples tested (0.03 to 0.25 μg/ml) and reduced the inoculum by ≥3 $\log_{10}$ as early as 4 to 8 h against MRSA MED 1805 and MRSA MED 2028. In contrast, vancomycin at 8× MIC required 24 h to reach the bactericidal endpoint against all three strains. Linezolid was bacteriostatic against the MRSA strains. Against VISA Mu50, TD-1792 was bactericidal at all MIC multiples with 0.12 μg/ml (2× MIC) reducing the inoculum by >3 $\log_{10}$ by 8 h. At 0.25 and 0.5 μg/ml, TD-1792 was bactericidal as early as 4 h. When tested at 8× MIC, vancomycin and linezolid were bacteriostatic at 24 h.

Postantibiotic effect (PAE) was determined according to the method outlined by Craig and Gudmundsson (5). After a 1-h exposure to TD-1792 at 4× MIC, growth of *S. aureus* ATCC 29213 (MIC = 0.015 μg/ml) and ATCC 33591 (MIC = 0.03 μg/ml) was suppressed for 2.2 h and 2.7 h, respectively. The PAEs of TD-1792 were similar to those observed for the comparators; vancomycin (for both strains, 2.4 to 3.4 h), nafcillin (for ATCC 29212, 2.1 h), or linezolid (for ATCC 33591, 3.2 h).

Comparison of the stability of TD-1792 to staphylococcal β-lactamases is shown in Table 3. As expected, there was no more than a 2-fold difference in the TD-1792 MIC between the β-lactamase-negative strain and strains producing various staphylococcal β-lactamase types. These results were consistent with the stability of THRX-169797, representing the cephalosporin moiety of TD-1792.

Our results demonstrate the potent *in vitro* inhibitory activity of TD-1792 against *S. aureus* isolates, including the emerging hVISA phenotype. Based upon MIC_{90} comparisons, TD-1792 was consistently the most active antibiotic tested against the isolates profiled in this study. It is notable that the activity of THRX-169797, the cephalosporin component of the heterodimer, is modest against MSSA ATCC 29213 (MIC, 1 μg/ml) and is 8-fold less active against MRSA ATCC 33591 (MIC, 8 μg/ml). Neverthe-

TABLE 2 Kill kinetics of TD-1792 and comparators against six *S. aureus* isolates

Organism	Antibiotic	MIC (μg/ml)	Concn tested (μg/ml)	$\Delta\log_{10}$ CFU/ml at:			
				2 h	4 h	8 h	24 h
MSSA ATCC 29213	TD-1792	0.015	0.03	−2.1	−3.0	−3.9	−4.3
			0.06	−0.8	−2.4	−3.9	−4.7
			0.12	−0.5	−2.4	−4.2	−3.8
	Vancomycin	1	8	−0.7	−0.9	−2.6	−4.7
	Nafcillin	0.5	4	−0.6	−1.0	−2.5	−4.6
	Cefazolin	0.5	4	0.1	−0.5	−2.4	−3.0
MSSA ATCC 13709	TD-1792	0.015	0.03	−1.9	−3.4	−4.1	−5.5
			0.06	−1.5	−2.7	−3.4	−5.4
			0.12	−1.6	−3.5	−4.6	−5.3
	Vancomycin	0.5	4	−0.2	0.0	−1.0	−5.0
	Nafcillin	1	8	−0.2	−0.1	−0.9	−5.7
	Cefazolin	0.5	4	0.2	−0.4	−2.3	−3.2
MRSA ATCC 33591	TD-1792	0.03	0.06	0.1	−0.3	−1.5	−4.2
			0.12	−0.1	−0.3	−1.4	−4.1
			0.25	−0.7	−1.2	−2.3	−3.7
	Vancomycin	1	8	0.0	0.1	−0.9	−3.2
	Linezolid	1	8	0.1	0.1	−0.8	−1.7
MRSA MED 1805	TD-1792	0.015	0.03	−0.5	−2.7	−3.2	−3.3
			0.06	−1.0	−2.6	−3.2	−4.2
			0.12	−1.9	−3.1	−3.7	−3.9
	Vancomycin	0.5	4	−0.1	−0.2	−0.9	−3.4
	Linezolid	2	16	0.0	−0.4	−0.3	−1.6
MRSA MED 2028[a]	TD-1792	0.03	0.06	−0.2	−3.3	−3.9	−3.7
			0.12	−0.7	−3.3	−3.8	−4.4
			0.25	−3.6	−3.7	−4.2	−4.3
	Vancomycin	1	8	0.1	−0.1	−0.7	−4.0
	Linezolid	2	16	0.0	−0.6	−1.8	−2.7
VISA Mu50	TD-1792	0.06	0.12	−0.7	−2.4	−3.3	−3.6
			0.25	−1.5	−3.2	−4.1	−4.2
			0.5	−2.3	−4.0	−4.2	−4.1
	Vancomycin	4	32	−0.2	−0.4	−0.8	−2.5
	Linezolid	2	16	−0.2	−0.3	−0.9	−2.3

[a] MRSA isolate nonsusceptible to daptomycin.

less, due to its unique chemical construct, the antistaphylococcal activity of TD-1792 is unaffected by coexisting resistance mechanisms, including resistance to methicillin/oxacillin and heterogeneous resistance to vancomycin. This finding suggests a cooperative mechanism of action between the cephalosporin and glycopeptide components of TD-1792 and warrants further study.

In a phase 1 study, administration of a single dose of TD-1792 intravenously at 2 mg/kg of body weight yielded plasma concentrations that exceed the MIC at which 100% of MRSA

TABLE 3 Susceptibility of β-lactamase-producing staphylococci to TD-1792

Strain	β-lactamase		MIC (μg/ml)		
	Type	Level[a]	TD-1792	THRX-169797[b]	Penicillin G
ATCC 25923	Negative	0	0.015	1	0.03
ATCC 29213	A	0.006	0.015	1	1
PC1	A	0.201	0.015	1	256
NCTC9789	A	0.088	0.015	1	64
22260	B	0.024	0.015	2	16
V137	C	0.034	0.03	2	64
FAR19	D	0.030	0.015	1	2

[a] Activity reported as micromoles of nitrocefin degraded/min/cell mass after incubation (8).
[b] THRX-169797 is the cephalosporin moiety of TD-1792.

isolates are inhibited (0.06 μg/ml) for 24 h (15). At this dose, serum concentrations are predicted to exceed the AUC/MIC target ratio required for efficacy *in vivo* (7). This survey of the susceptibilities of multidrug-resistant *S. aureus* isolates to TD-1792, together with the favorable *in vitro* pharmacodynamic interactions described herein, supports the continued development of this new agent for the treatment of serious infections caused by *S. aureus*.

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