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In Vitro Activity of TD-6424 against *Staphylococcus aureus*

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TD-6424, a rapidly bactericidal agent with multiple mechanisms of action, is more potent in vitro and more rapidly bactericidal than currently available agents against methicillin-susceptible and methicillin-resistant *Staphylococcus aureus*. TD-6424 produces a postantibiotic effect with a duration of 4 to 6 h against these organisms. The results suggest potential efficacy against susceptible and resistant strains of *S. aureus*.

Drug-resistant *Staphylococcus aureus* has become an increasingly important problem for the management of serious infections (2, 3, 6). TD-6424 is a new agent which is rapidly bactericidal for gram-positive bacteria. Its activity is mediated via a pair of biochemically distinct mechanisms. In addition to inhibition of peptidoglycan synthesis, TD-6424 also inhibits the synthesis of phospholipids required for integrity of the bacterial cell membrane (J. L. Pace, Abstr. 42nd Intersci. Conf. Antimicrob. Agents Chemother., abstr. 454, p. 614, 2002). In the present study, the susceptibilities of methicillin-susceptible *S. aureus* (MSSA), methicillin-resistant *S. aureus* (MRSA), and glycopeptide-intermediate *S. aureus* (GISA) to TD-6424, vancomycin, oxacillin, linezolid, and teicoplanin were evaluated. The bactericidal activities and postantibiotic effects (PAEs) of TD-6424 and vancomycin against MSSA, MRSA, and GISA were also assessed.

S. aureus strains were clinical isolates obtained within the last 4 years from a variety of hospitals in the United States and Europe. Well-characterized *S. aureus* strains from the American Type Culture Collection (ATCC; Manassas, Va.), the Centers for Disease Control and Prevention (Atlanta, Ga.), and other sources were also used. TD-6424 was obtained from Theravance, and all other agents were obtained from Sigma-Aldrich (St. Louis, Mo.), the U.S. Pharmacopeia (Rockville, Md.), or Hoechst AG (now Aventis; Frankfurt am Main, Germany).

The MIC was determined by the broth microdilution method according to protocol M7-A5 of the NCCLS (5). The minimum bactericidal concentration (MBC) was determined according to protocol M26-A of the NCCLS (4) from the microtiter plates used to determine MICs with a starting inoculum of $\sim 5 \times 10^5$ CFU/ml. After a 24-h incubation, the number of survivors from wells with no obvious turbidity was determined by plate counting of 10-μl samples. The bactericidal activity was determined by a time-kill assay (4) in 10 ml of Mueller-Hinton broth with a starting inoculum of $\sim 10^6$ CFU/ml of log-phase cultures. At regular intervals, bacteria were enumerated by plate counting from 200 μl samples. For both MBC and time-kill assays, bactericidal activity was defined as a ≥ 3-$\log_{10}$ decrease in the number of CFU per milliliter within 24 h. The limit of detection was 10 CFU/ml.

The durations of the PAEs were determined according to a published protocol (1) with the following modifications. In brief, the 20-ml starting inoculum of $\sim 10^6$ CFU/ml was exposed to the MIC of the compound for 1 h at 37°C. Bacteria were then removed from the antibiotic-containing medium by centrifugation and washed with saline to remove residual antibiotic. Bacteria were resuspended in 20 ml of prewarmed Mueller-Hinton broth and incubated with shaking. Plate counts were performed from 100-μl samples diluted appropriately at hourly intervals to determine the time at which a 1-$\log_{10}$ increase in CFU per milliliter occurred after antibiotic removal. The duration of PAE was calculated as the number of hours until return to log-phase growth of treated bacteria minus the time for return of control bacteria (no antibiotic but treated identically) to log-phase growth.

Susceptibility testing. TD-6424 was highly active against MSSA. The MICs of TD-6424 for these organisms were generally lower than those of vancomycin, oxacillin, linezolid, and teicoplanin (Table 1). The MICs of TD-6424 for 47 strains of MSSA ranged from 0.12 to 2 μg/ml, and the MIC at which 90% of organisms were inhibited (MIC_{90}) was 1 μg/ml. The MIC of TD-6424 for ATCC 13709 was 0.5 μg/ml, compared to 0.5 μg/ml for nafcillin, gentamicin, and oxacillin, 1 μg/ml for vancomycin and teicoplanin, and 4 μg/ml for linezolid. TD-6424

TABLE 1. In vitro activities of TD-6424 and comparator antibiotics against MRSA and MSSA

Organism	No. of strains	Antimicrobial agent	MIC (μg/ml)		
			Range	50%	90%
MRSA	128	TD-6424	≤0.06–2	1	1
	128	Vancomycin	0.5–2	1	2
	39	Teicoplanin	0.5–16	1	4
	128	Linezolid	0.5–4	2	4
	128	Oxacillin	4–>64	64	>64
MSSA	47	TD-6424	0.12–2	0.5	1
	47	Vancomycin	0.5–2	2	2
	38	Teicoplanin	0.5–2	1	2
	47	Linezolid	2–4	2	2
	47	Oxacillin	0.13–2	0.5	1

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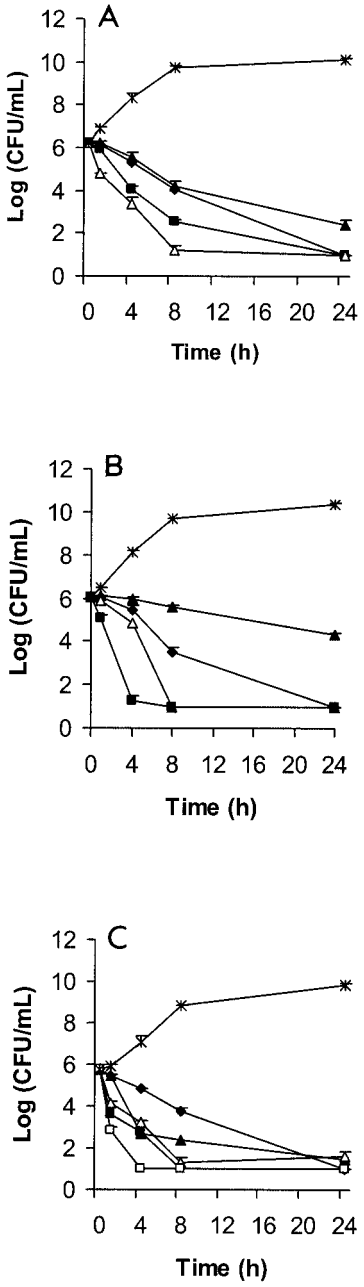

FIG. 1. Time-kill curves for MSSA (ATCC 13709) (A), MRSA (ATCC 33591) (B), and GISA (HIP 5836) (C). (A) Vancomycin at 4 times the MIC (◆), nafcillin at 4 times the MIC (■), linezolid at 4 times the MIC (▲), and TD-6424 at 4 times the MIC (△); (B) vancomycin at 8 times the MIC (◆), linezolid at 8 times the MIC (▲), and TD-6424 at 8 (△) and 32 (■) times the MIC; (C) linezolid at 4 times the MIC (▲), vancomycin at 4 times the MIC (◆), and TD-6424 at 4 (△), 8 (■), and 16 (□) times the MIC. ∗, bacterial growth control. The nafcillin MIC was 1 μg/ml for ATCC 13709. Vancomycin MICs were 1, 1, and 8 μg/ml for ATCC 13709, ATCC 33591, and HIP 5836, respectively; the corresponding values were 2, 2, and 2 μg/ml for linezolid and 1, 1, and 2 μg/ml for TD-6424.

TABLE 2. PAE for TD-6424 and comparator antibiotics

Antibiotic	PAE (h)		
	MSSA (ATCC 13709)	MRSA (ATCC 33591)	GISA (HIP 5836)
TD-6424	4	6	4
Vancomycin	1	1	1
Nafcillin	0	ND[a]	ND

[a] ND, not determined.

was more active against MRSA than were vancomycin, oxacillin, linezolid, and teicoplanin (Table 1). The MICs of TD-6424 for 128 strains of MRSA ranged from ≤0.06 to 2 μg/ml, and the MIC_{90} was 1 μg/ml. The MIC of TD-6424 for ATCC 33591 was 0.5 μg/ml, compared to 1 μg/ml for vancomycin, 2 μg/ml for teicoplanin, linezolid, and gentamicin, and 128 μg/ml for nafcillin and oxacillin. TD-6424 (MIC 2 μg/ml) was more active than vancomycin, teicoplanin, nafcillin, and oxacillin (all MICs, 8 μg/ml) against a well-characterized strain of GISA (HIP 5836).

Bactericidal activity. The MBCs of TD-6424 for MSSA (ATCC 13709), MRSA (ATCC 33591), and GISA (HIP 5836) were 2, 4, and 4 μg/ml, respectively; the corresponding values for vancomycin were 2, 4, and 8 μg/ml, and those for nafcillin were 0.5, >128, and 128 μg/ml. The results of time-kill assays demonstrated the rapid, concentration-dependent bactericidal activity of TD-6424. Results for MSSA indicated that TD-6424 (4 μg/ml) reduced the initial inoculum from $\log_{10}$ 6.22 ± 0.08 CFU/ml to $\log_{10}$ 3.36 ± 0.28 CFU/ml within 4 h after administration (Fig. 1A). Generally, similar results were obtained for nafcillin (4 μg/ml). With vancomycin (4 μg/ml), the initial inoculum was decreased by only about 1 $\log_{10}$ at 4 h. Linezolid (8 μg/ml) exhibited the least bactericidal activity in this assay. Results with TD-6424 for MRSA were generally similar to those for MSSA (Fig. 1B): TD-6424 at 8 and 32 μg/ml reduced the initial inoculum from $\log_{10}$ 6.05 ± 0.08 CFU/ml to $\log_{10}$ 4.87 ± 0.05 and 1.26 ± 0.26 CFU/ml, respectively, within 4 h. Vancomycin at 8 μg/ml exhibited much slower bactericidal activity, and linezolid at 16 μg/ml had almost no bactericidal activity against this organism. TD-6424 at 8, 16, and 32 μg/ml was also rapidly bactericidal against GISA (Fig. 1C). By 4 h, the 8- and 32-μg/ml concentrations had reduced the initial inoculum from $\log_{10}$ 5.76 ± 0.07 to 3.21 ± 0.12 and 0.98 ± 0.02 CFU/ml, respectively. Linezolid at 8 μg/ml reduced the initial inoculum from $\log_{10}$ 5.76 ± 0.07 CFU/ml to $\log_{10}$ 2.73 ± 0.08 CFU/ml by 4 h, and vancomycin at 32 μg/ml decreased it from $\log_{10}$ 5.76 ± 0.07 CFU/ml to $\log_{10}$ 4.82 ± 0.02 CFU/ml over this period.

PAE. TD-6424 had long durations (≥4 h) of PAE against MSSA, MRSA, and GISA (Table 2). Vancomycin had substantially shorter PAEs, whereas nafcillin did not generate a PAE against the MSSA strain in this assay.

In summary, TD-6424 is highly active against MSSA, MRSA, and GISA and has significant bactericidal activity and a long PAE against these organisms. The in vitro properties of TD-6424 suggest that it may be effective in infections caused by both susceptible and resistant strains of *S. aureus*.

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