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In Vitro Activity of LY333328, an Investigational Glycopeptide Antibiotic, against Enterococci and Staphylococci

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The in vitro activities of LY333328 were compared with those of vancomycin, teicoplanin, and quinupristin-dalfopristin (Synercid) against 219 strains of enterococci and staphylococci, including vancomycin-resistant enterococci and methicillin-resistant *Staphylococcus aureus*. MICs and MBCs were determined by a microtiter dilution protocol. LY333328 demonstrated superior activity against vancomycin-resistant enterococci and was the only antibiotic which was bactericidal. Its potency was comparable or superior to those of other antibiotics tested against methicillin-resistant staphylococci.

Enterococci and staphylococci are the most prevalent bacteria recovered from patients with nosocomial infections (15). The expression of intrinsic and acquired antibiotic resistance determinants is a major contributor to the persistence of these organisms. Staphylococci are frequently resistant to the penicillinase-stable penicillins, leaving vancomycin as the sole drug available for treatment of serious staphylococcal infections (4). The emergence of enterococci resistant to vancomycin has resulted in a subset of clinical isolates that cannot be treated with any currently available antibiotic (8).

LY333328 is a semisynthetic glycopeptide that has reported activity against gram-positive bacteria, including methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) (13). The chemical structure of LY333328 is shown in Fig. 1. The objective of the current study was to compare in vitro inhibitory and bactericidal activities of LY333328 with those of vancomycin, teicoplanin, and the streptogramin antibiotic quinupristin-dalfopristin (Synercid).

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Enterococci and methicillin-resistant strains of staphylococci were recent clinical isolates recovered from the Clinical Microbiology Laboratories at the University of Maryland Medical Systems and the Medical Center of Delaware. Staphylococci were identified to species level with either API STAPH (bioMerieux Vitek, Inc., Hazelwood, Mo.) (University of Maryland Medical Systems) or Vitek (bioMerieux Vitek, Inc.) (Medical Center of Delaware). Staphaurex (Murex Diagnostics Ltd., Dartford, England) was used to confirm the identity of *S. aureus*. Resistance to methicillin was confirmed with MRSA Screen Agar (Becton Dickinson Microbiology Systems, Cockeysville, Md.). The biochemical schema of Facklam and Collins as well as their criteria for motility and pigment production was used for identification of enterococci to species level (3). Resistance to vancomycin was confirmed with an E test strip (AB Biodisk, Solna, Sweden). A subset of 31 vancomycin-resistant *Enterococcus faecium* and *Enterococcus faecalis* strains used in this study were genetically typed to ensure that *vanA* and *vanB* strains were included. PCR methods and primers were the same as those described previously (7). Interpretations of susceptibilities, with the exception of those of enterococci to teicoplanin and vancomycin, were based upon recommendations of the National Committee for Clinical Laboratory Standards (12). For this study, vancomycin and teicoplanin resistance was defined as MICs of $\geq$16 μg/ml for *E. faecium* and *E. faecalis* strains. Teicoplanin resistance in enterococci has similarly been defined as an MIC of $\geq$16 μg/ml (1).

LY333328 powder was supplied by Eli Lilly Research Laboratories, Indianapolis, Ind. Teicoplanin powder was a gift from Hoechst Marion Roussel Research Institute, Hoechst Marion Roussel, Inc., Cincinnati, Ohio. Quinupristin-dalfopristin powder was a gift from Rhone-Poulenc Rorer Pharmaceuticals Inc., Collegeville, Pa. The ratio of quinupristin to dalfopristin was 3:7. Vancomycin was purchased from Sigma Laboratories, St. Louis, Mo. All antibiotics were reconstituted according to the manufacturers' instructions.

A microtiter dilution procedure was used to determine MICs. For the in vitro protocol, we followed the recommendations of the National Committee for Clinical Laboratory Standards (11). The final concentrations for each antibiotic except vancomycin ranged from 0.12 to 128 μg/ml in a total volume of 200 μl. Vancomycin concentrations were increased to 1,024 μg/ml. The final organism concentration (as determined by colony counts) ranged from 1×10^5 to 5×10^5 CFU/ml. Quality control strains *E. faecalis* ATCC 29212 and *S. aureus* ATCC 29213 were included with each test run to ensure potency. The MIC was defined as the concentration at which the first visually clear well appeared after incubation at 35°C for 24 h.

Prior to performing MBC testing, we placed microtiter plates on a rotating apparatus after 20 h of incubation in order to dislodge any bacteria adhering to the plastic. Following an additional 4 h of incubation, 100 μl (one-half of the total volume) was removed from each well and plated on Trypticase soy-sheep blood agar and incubated for 24 h at 35°C. A separate microtiter tip was used for each microtiter dilution in

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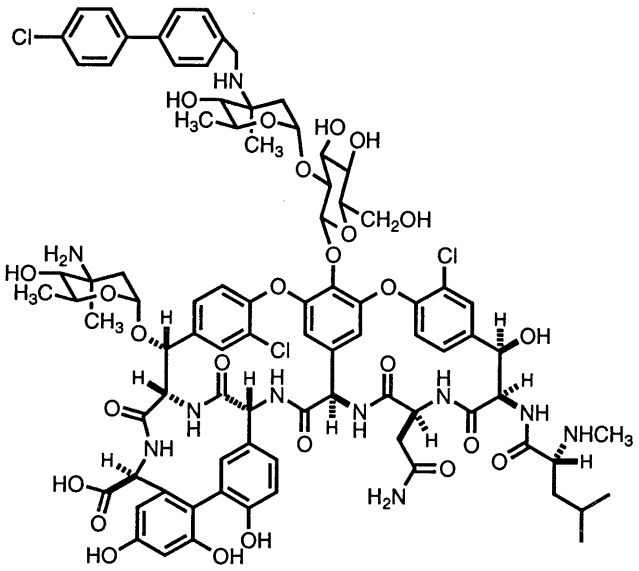

FIG. 1. Chemical structure of LY333328.

order to minimize antibiotic carryover. CFU from each plate were then enumerated. The MBC was defined as the first dilution which resulted in a ≥99.9% decrease from the initial bacterial titer of the starting inoculum.

Time-kill curves were performed by a saline dilution procedure described previously (16). *E. faecium* (*vanA*) was exposed to LY333328 at the MIC, four times the MIC, and the MBC; vancomycin and LY333328 activities were compared for a strain of MRSA at the MIC and MBC. Samples were removed at 0, 3, 6, and 24 h and diluted 10^{-1} to 10^{-6} in saline. One hundred microliters of the 10^{-1} dilution was plated, and 10 μl of each of the 10^{-2} to 10^{-6} dilutions was spotted on a brain heart infusion agar plate and incubated for 72 h at 35°C prior to the counting of colonies.

Thirty-one vancomycin-resistant *E. faecium* and *E. faecalis* stains were screened by PCR for genes which encoded vancomycin resistance. Twelve strains had the *vanA* genotype, and 19 strains had the *vanB* genotype. LY333328 inhibited all strains tested at concentrations of ≤2 μg/ml (Table 1). MICs for VRE were generally two- to fourfold higher than those for vancomycin-susceptible enterococcal strains. LY333328 was also active against all methicillin-resistant staphylococcal strains tested, including strains exhibiting a double zone of inhibition to imipenem. These strains have previously been shown by our laboratory to express increased levels of resistance to vancomycin (16). LY333328 inhibited the quality control strains *S. aureus* ATCC 29213 and *E. faecalis* 29212 at concentrations of 0.5 to 1 and 0.25 to 0.5 μg/ml, respectively.

As previously described, quinupristin-dalfopristin demonstrated good inhibitory activity against the majority of vancomycin-resistant and -susceptible strains of *E. faecium* (2). Quinupristin-dalfopristin was less effective against *Enterococcus gallinarum* and was unsatisfactory against the majority of *E. faecalis* isolates, confirming a previous report (2). The in-

TABLE 1. In vitro activity of LY333328

Organism (no. of isolates)	Compound	MIC (μg/ml)[a]			MBC (μg/ml)[a]		
		Range	50%	90%	Range	50%	90%
E. faecium, vancomycin resistant (51)	LY333328	≤0.12–2	0.5	1	2–16	8	16
	Quin-dal[b]	≤0.12–4	1	2	0.5–>128	8	>128
	Teicoplanin	≤0.012–128	32	64	4–>128	>128	>128
	Vancomycin	32–>1,024	512	>1,024	64–>1,024	>1,024	>1,024
E. faecium, vancomycin susceptible (30)	LY333328	≤0.12–1	0.25	0.5	1–8	2	4
	Quin-dal	0.5–8	4	8	2–128	16	64
	Teicoplanin	≤0.012–4	0.5	2	1–128	16	64
	Vancomycin	0.5–4	1	4	2–>128	64	>128
E. faecalis vancomycin resistant (30)	LY333328	≤0.12–2	0.5	2	4–16	8	16
	Quin-dal	2–32	16	32	8–>128	>128	>128
	Teicoplanin	≤0.012–128	0.5	32	1–>128	32	>128
	Vancomycin	16–1,024	512	1,024	128–>1,024	1,024	>1,024
E. faecalis, vancomycin susceptible (30)	LY333328	≤0.12–2	0.5	1	2–8	4	8
	Quin-dal	2–64	16	32	8–>128	64	>128
	Teicoplanin	≤0.12–2	0.25	1	1–128	16	64
	Vancomycin	0.25–4	1	4	32–>128	128	>128
E. gallinarum (11)	LY333328	≤0.12–1	0.5	0.5	0.25–8	2	4
	Quin-dal	1–16	4	8	4–>128	8	16
	Teicoplanin	≤0.12–4	0.5	2	8–>128	64	128
	Vancomycin	4–16	8	16	128–>1,024	1,024	>1,024
S. aureus methicillin resistant (21)	LY333328	0.25–2	0.5	1	0.25–4	1	2
	Quin-dal	≤0.12–4	1	2	≤0.12–>128	16	>128
	Teicoplanin	≤0.12–4	1	2	0.25–4	1	4
	Vancomycin	0.5–2	1	2	0.5–4	1	2
Staphylococcus epidermidis, methicillin resistant (21)	LY333328	≤0.12–1	0.5	1	≤0.12–2	1	2
	Quin-dal	≤0.12–2	0.5	1	≤0.12–64	4	8
	Teicoplanin	≤0.12–8	4	8	≤0.12–16	8	16
	Vancomycin	0.25–4	1	2	1–4	2	4
S. haemolyticus methicillin resistant (25)	LY333328	≤0.12–2	1	2	≤0.12–4	1	2
	Quin-dal	≤0.12–2	1	2	0.25–8	2	4
	Teicoplanin	2–128	16	128	2–>128	32	>128
	Vancomycin	1–8	2	8	0.5–16	4	8

[a] 50% and 90%, MICs or MBCs at which 50 and 90% of the isolates are inhibited or killed.
[b] Quin-dal, quinupristin-dalfopristin (30:70).

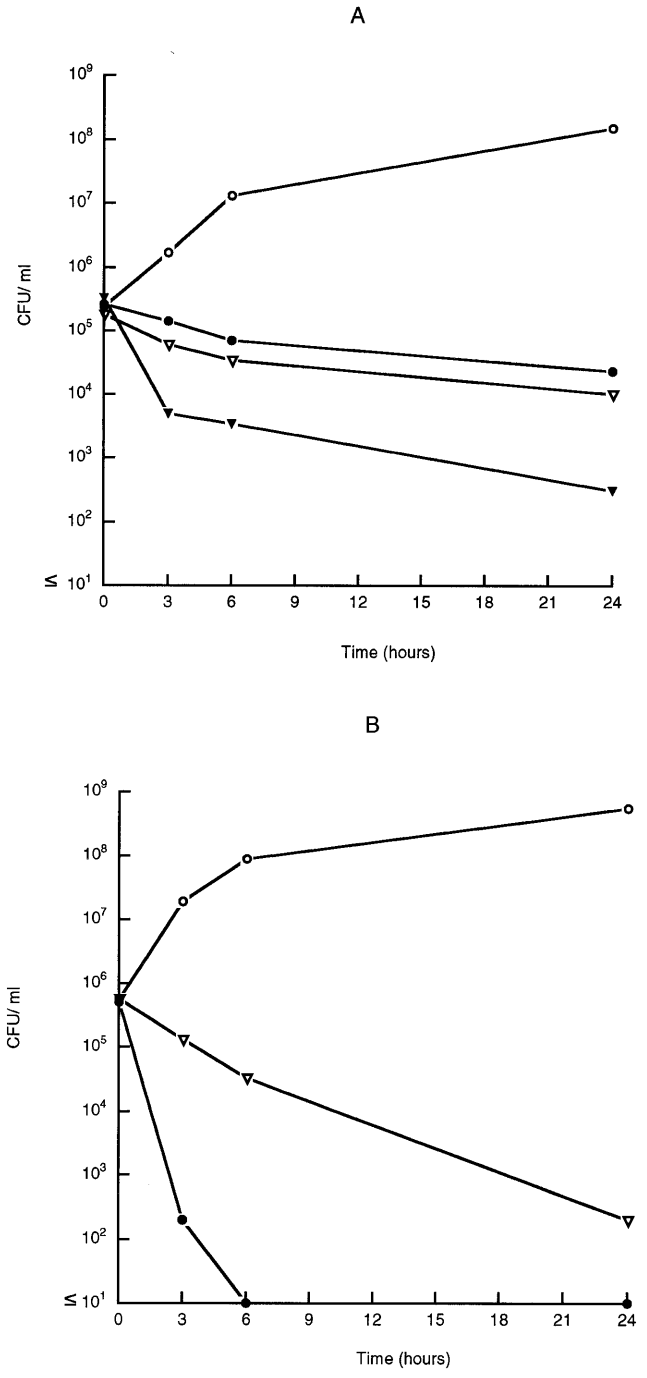

FIG. 2. Representative time-kill curves. (A) Vancomycin-resistant *E. faecium* (*vanA*) organisms were not treated to allow growth (control) (○) or were treated with 1 μg of LY333328 per ml (●), 4 μg of LY333328 per ml (△), or 16 μg of LY333328 per ml (▲). The LY333328 MIC and MBC were 1 and 16 μg/ml, respectively. (B) MRSA organisms were not treated to allow growth (control) (○) or were treated with 2 μg of LY333328 per ml (●) or 2 μg of vancomycin per ml (△). The LY333328 and vancomycin MICs and MBCs were 2 μg/ml.

hibitory activity of this agent against methicillin-resistant staphylococci was similar to that of LY333328.

Teicoplanin did not inhibit the majority of the VRE tested. All 12 *vanA* strains were resistant to teicoplanin (MICs, ≥16 μg/ml). Although *vanB* strains were initially described to be susceptible to teicoplanin, DNA prepared from 8 of 19 teicoplanin-resistant enterococci hybridized to and were amplified solely by the *vanB* PCR primers. This observation supports recent reports made by other investigators suggesting that the *vanA* genotype cannot be predicted by phenotypic resistance to teicoplanin (5, 18). Teicoplanin failed to inhibit 18 of 25 methicillin-resistant strains of *Staphylococcus haemolyticus* (MICs, ≥16 μg/ml) as has been previously described (10, 16). Vancomycin also was less active against *S. haemolyticus* strains. Three of four imipenem double-zone strains tested required MICs of vancomycin of 8 μg/ml, which was similar to what has been demonstrated previously (16).

LY333328 was the only compound tested which was bactericidal for VRE as well as vancomycin-susceptible enterococci (Table 1). The MBC results for enterococci ranged from 1 to 16 μg/ml and were generally higher for vancomycin-resistant strains than for susceptible isolates. MBCs for enterococci exceeded the MICs by 2- to 64-fold and required 48 to 72 h of incubation for accurate interpretation, perhaps because of partial inhibition by antibiotic carryover. Conversely, LY333328 MBCs for staphylococci were equal to or 1 twofold dilution higher than the corresponding MICs and the values did not increase after 24 h of incubation. Kill-curve kinetics confirmed the MIC and MBC results (Fig. 2). Concentrations of LY333328 16-fold higher than those for inhibitory activity were required to demonstrate a 3-$\log_{10}$-unit decrease in viable colonies of VRE after 24 h of exposure. The MRSA test strain was rapidly killed (within 3 h) at the LY333328 MIC. The other three test antibiotics had minimal to no bactericidal activities against enterococci. Quinupristin-dalfopristin also failed to kill 8 of the 21 tested MRSA strains (MBCs, 64 to >128 μg/ml). Lack of bactericidal activity of this agent against some MRSA strains has been reported (6, 9). Pulsed-field gel electrophoresis results suggested that these isolates were not clonal in origin (data not shown).

In summary, LY333328 appears to have promising in vitro activity. It is derived from the same parent glycopeptide (LY264826) as the recently described semisynthetic compound LY191145 (14). However, LY333328 has superior inhibitory activity for tested vancomycin-resistant strains of enterococci. The bactericidal activity displayed by this agent against all tested strains of enterococci, including VRE, is unique. It is important to note, however, that increased concentrations of LY333328 (relative to inhibitory concentrations) were required for bactericidal activity and 72 h of incubation was necessary for accurate interpretation. Recent experiments in our laboratory using enterococcal *vanB* strains suggest that addition of subinhibitory concentrations of vancomycin does not induce resistance to LY333328 (data not shown). There is a paucity of agents currently available for treatment of disease caused by multiply antibiotic-resistant strains of enterococci and staphylococci. The results of this study suggest that LY333328 should be further characterized in experimental animal models to accumulate safety and pharmacokinetic data.

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