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In Vitro Antibacterial Activity of LY333328, a New Semisynthetic Glycopeptide

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LY333328 is a semisynthetic *N*-alkyl derivative of LY264826, a naturally occurring structural analog of vancomycin. LY333328 was evaluated for its in vitro inhibitory and bactericidal activities in comparison with those of the two currently available glycopeptides (vancomycin and teicoplanin). Glycopeptide-susceptible test strains included a total of 311 isolates (most of clinical origin) from the genera *Staphylococcus*, *Enterococcus*, *Streptococcus*, *Aerococcus*, *Gemella*, *Lactococcus*, *Listeria*, *Corynebacterium*, and *Clostridium*. Test strains resistant or intermediate to vancomycin and/or teicoplanin included 56 clinical isolates of *Enterococcus* (of the VanA, VanB, and VanC phenotypes) and 32 clinical isolates of *Staphylococcus* (*S. haemolyticus*, *S. epidermidis*, and *S. aureus*), 31 strains of gram-positive genera outside the spectrum of activity of vancomycin (*Leuconostoc*, *Pediococcus*, *Lactobacillus*, and *Erysipelothrix*), and laboratory-derived organisms obtained after exposure of susceptible *Staphylococcus* isolates to teicoplanin (6 strains) or laboratory-derived organisms with resistance determinants received from VanA enterococci (2 *Enterococcus* and 25 *Listeria* transconjugants). LY333328 was highly active against staphylococci, enterococci, and listeriae (whether they were clinical or laboratory-derived strains) resistant to the currently available glycopeptides. In particular, the MICs of LY333328 did not vary substantially between teicoplanin-susceptible and teicoplanin-resistant staphylococci and between vancomycin-susceptible and vancomycin-resistant enterococci. LY333328 demonstrated fairly good inhibitory activity even against most strains of *Leuconostoc*, *Pediococcus*, and *Erysipelothrix* (MIC range, 1 to 8 μg/ml), whereas it proved less active (although much more active than vancomycin or teicoplanin) against *Lactobacillus* strains. In minimal bactericidal concentration (MBC) and time-kill studies, LY333328 demonstrated excellent bactericidal activity; enterococci, in particular, which were largely tolerant of vancomycin and teicoplanin, were uniformly killed by LY333328, with MBC-to-MIC ratios of 4 to 8 for most vancomycin-susceptible and vancomycin-resistant strains. In attempts to select for resistant clones, no survivors stably growing in the presence of 10 μg of LY333328 per ml were obtained from the *Staphylococcus* and *Enterococcus* test strains exposed to the drug.

Among the pathogens within the spectrum of activity of glycopeptides, no trends toward resistance were noted during the first 30 years of clinical experience with vancomycin (9), and a similarly uniform activity appeared to be true of teicoplanin (40, 42), the other currently available glycopeptide. This was the case so much so that it had even been hypothesized that mutations leading to glycopeptide resistance would be lethal for the cell (32). Conversely, resistance to glycopeptides (i.e., to vancomycin or teicoplanin, or both, depending on the organisms) suddenly and unexpectedly emerged in the late 1980s, at about the same time, among coagulase-negative staphylococci (1, 33, 43) and enterococci (16, 37). Vancomycin resistance in enterococci, in particular, is encoded by genes usually located on mobile elements and is transferable to susceptible gram-positive recipients (2, 15, 17, 30, 35). The potential for the spread of vancomycin resistance to other organisms is illustrated by the recent finding of the *vanA* determinant in previously noninvolved *Enterococcus* species (11) and in *Corynebacterium*, *Arcanobacterium*, *Oerskovia*, *Lactococcus* (14), and *Bacillus* (13) species and of a *vanB*-related gene in a fecal isolate of *Streptococcus bovis* (31). Nosocomial infections caused by vancomycin-resistant enterococci are increasingly reported in the United States, where they are becoming an alarming health problem (7, 21), and less frequently in Europe (5, 19, 38, 41). Acquired vancomycin resistance in enterococci is often associated with resistance to multiple antibiotics, and infections caused by these organisms are sometimes not treatable with any currently available antibiotic or antibiotic combination (20, 21, 36).

The emergence of glycopeptide resistance is contributing significantly to the growing concern about the impact of antimicrobial resistance on therapeutic options (8, 20, 34). Indeed, the development of new molecules capable of overcoming the emerging resistances to currently available glycopeptides is now a very topical and widely recognized need. Promising data have been reported with certain semisynthetic derivatives of vancomycin (22, 27) and LY264826 (10, 23, 28, 29), a naturally occurring vancomycin analog formerly referred to as A82846B (22, 23). In the present study, LY333328, a semisynthetic N-alkylated derivative of LY264826 containing a chlorodiphenyl side chain (10), was evaluated in vitro for its inhibitory and bactericidal activities against a variety of gram-positive organisms susceptible or resistant to the available glycopeptides. In particular, test strains resistant or intermediate to vancomycin

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TABLE 1. Comparative activities (MICs and MBCs) of LY333328, vancomycin, and teicoplanin against 149 staphylococci

Organism group and organism (no. of isolates tested)	Antimicrobial agent	MIC (μg/ml)[a]			MBC (μg/ml)[b]		
		Range	50%	90%	Range	50%	90%
Randomly collected clinical isolates							
Staphylococcus aureus oxacillin-susceptible (12)	LY333328	1–4	2	4	2–16	8	8
	Vancomycin	0.25–2	1	2	0.5–8	2	8
	Teicoplanin	0.125–4	0.5	4	0.25–16	2	8
Staphylococcus aureus, oxacillin-resistant (27)	LY333328	1–4	2	4	2–16	8	16
	Vancomycin	0.25–4	1	2	0.5–8	2	4
	Teicoplanin	0.125–8	2	4	0.5–16	8	16
Staphylococcus epidermidis, oxacillin-susceptible (13)	LY333328	≤0.03–4	2	4	0.125–8	4	8
	Vancomycin	0.25–2	1	2	1–8	2	4
	Teicoplanin	≤0.03–4	1	4	1–8	2	8
Staphylococcus epidermidis, oxacillin-resistant (19)	LY333328	1–8	2	4	4–16	8	16
	Vancomycin	0.5–4	1	2	1–16	2	4
	Teicoplanin	0.125–16	4	8	1–32	8	32
Staphylococcus haemolyticus, oxacillin-susceptible (11)	LY333328	0.5–8	2	4	2–16	4	8
	Vancomycin	0.5–4	1	2	0.5–8	2	4
	Teicoplanin	0.25–8	2	8	0.5–16	4	16
Staphylococcus haemolyticus, oxacillin-resistant (10)	LY333328	2–4	2	4	8–16	8	16
	Vancomycin	1–4	2	2	2–8	4	4
	Teicoplanin	1–16	4	4	2–16	8	16
Other coagulase-negative staphylococci (19)[c]	LY333328	1–4	2	4	8–16	8	8
	Vancomycin	0.5–2	1	2	1–4	2	4
	Teicoplanin	0.5–4	2	4	2–16	4	8
Strains selected as resistant or with reduced susceptibility to teicoplanin (MIC, ≥8 μg/ml)							
Staphylococcus aureus (6)	LY333328	2–4			4–16		
	Vancomycin	1–2			2–4		
	Teicoplanin	8–16			8–32		
Staphylococcus epidermidis (12)	LY333328	1–4	2	4	4–32	8	8
	Vancomycin	1–4	1	2	1–16	4	8
	Teicoplanin	8–32	8	16	16–64	16	32
Staphylococcus haemolyticus (20)	LY333328	1–8	4	4	8–32	8	16
	Vancomycin	1–8	2	4	4–32	4	8
	Teicoplanin	8–128	32	128	16–>256	64	256

[a] 50% and 90%, MICs at which 50 and 90% of isolates tested are inhibited, respectively.
[b] 50% and 90%, MBCs at which 50 and 90% of isolates tested are inhibited, respectively.
[c] Four strains of *S. simulans*, three strains of *S. capitis*, three strains of *S. hominis*, three strains of *S. saprophyticus*, two strains of *S. xylosus*, and one strain each of *S. auricularis*, *S. cohnii*, *S. lugdunensis*, and *S. warneri*.

and/or teicoplanin included clinical isolates of *Enterococcus* (of the VanA, VanB, and VanC phenotypes) and *Staphylococcus* (*S. haemolyticus*, *S. epidermidis*, and *S. aureus*); representatives of inherently glycopeptide-resistant gram-positive genera such as *Leuconostoc*, *Pediococcus*, *Lactobacillus*, and *Erysipelothrix*; *Enterococcus* and *Listeria* transconjugants which had directly or indirectly received glycopeptide resistance determinants from VanA enterococci under laboratory conditions (2); and laboratory-derived staphylococci obtained after exposure of susceptible strains to teicoplanin. Attempts to select for resistance to LY333328 were also performed with some *Staphylococcus* and *Enterococcus* isolates.

(Part of these data were presented at the 36th Interscience Conference on Antimicrobial Agents and Chemotherapy, New Orleans, La., 15 to 18 September 1996.)

MATERIALS AND METHODS

Antimicrobial agents. Vancomycin and teicoplanin were obtained from Eli Lilly Italia, Sesto Fiorentino, Italy, and the Marion Merrel Dow Research Institute, Gerenzano, Italy, respectively. LY333328 was supplied by Lilly Research Laboratories, Indianapolis, Ind., as a diphosphate salt, in the form of a lyophilized water-soluble powder. Penicillin and oxacillin were purchased from Sigma Chemical Co., St. Louis, Mo.

Bacterial strains. A total of 149 staphylococci, 120 enterococci, 68 streptococci, 10 strains of other catalase-negative vancomycin-susceptible gram-positive cocci, 47 listeriae, 12 corynebacteria, 24 clostridia, 20 lactic acid bacteria of inherently vancomycin-resistant genera, and 11 strains of *Erysipelothrix rhusiopathiae* were studied.

Staphylococci included 111 randomly collected clinical isolates; most were of the species *S. aureus*, *S. epidermidis*, and *S. haemolyticus*, and lower numbers were of the species *S. auricularis*, *S. capitis*, *S. cohnii*, *S. lugdunensis*, *S. hominis*, *S. saprophyticus*, *S. simulans*, *S. warneri*, and *S. xylosus*. A collection of 38 staphylococci selected on the basis of resistance or reduced susceptibility to teicoplanin (MICs, ≥8 μg/ml) included strains of *S. aureus*, *S. epidermidis*, and *S. hae-*

TABLE 2. Comparative activities (MICs and MBCs) of LY333328, vancomycin, and teicoplanin against 122 enterococci

Organism group (no. of isolates tested)	Antimicrobial agent	MIC (μg/ml)[a]			MBC (μg/ml)[b]		
		Range	50%	90%	Range	50%	90%
Vancomycin-susceptible isolates (64)[c]	LY333328	≤0.03–1	0.25	0.5	0.06–8	2	4
	Vancomycin	0.25–2	1	2	16–>256	64	256
	Teicoplanin	≤0.03–1	0.125	0.25	2–128	8	64
Strains of phenotype VanA (33)[d]	LY333328	0.06–1	0.5	1	1–16	4	8
	Vancomycin	64–>256	>256	>256	256–>256	>256	>256
	Teicoplanin	32–>256	64	>256	128–>256	>256	>256
Strains of phenotype VanB (5)[e]	LY333328	0.06–0.5			2–4		
	Vancomycin	4–8			64–>256		
	Teicoplanin	0.125–1			1–32		
Strains of phenotype VanC (20)[f]	LY333328	≤0.03–1	0.25	0.5	0.5–8	2	8
	Vancomycin	4–16	4	8	32–>256	128	>256
	Teicoplanin	0.125–4	0.125	0.5	4–>256	16	64

[a] See footnote *a* of Table 1.
[b] See footnote *b* of Table 1.
[c] Forty strains of *E. faecalis*, 14 strains of *E. faecium*, 3 strains of *E. durans*, 2 strains of *E. hirae*, and 1 strain each of *E. avium*, *E. maleodoratus*, *E. mundii*, *E. pseudoavium*, and *E. raffinosus*.
[d] Twenty-one strains of *E. faecium*, 10 strains of *E. faecalis*, and 1 strain each of *E. avium* and *E. durans*.
[e] Three strains of *E. faecium* and two strains of *E. faecalis*.
[f] Twelve strains of *E. casseliflavus*, five strains of *E. flavescens*, and three strains of *E. gallinarum*.

molyticus; 6 such strains (3 of *S. haemolyticus*, 2 of *S. epidermidis*, and 1 of *S. aureus*) were obtained from susceptible clinical parent strains exposed to teicoplanin, as described previously (3). On the basis of the results of standard broth microdilution tests (26), staphylococcal strains were preliminarily differentiated into oxacillin-susceptible (oxacillin MICs, ≤2 μg/ml) or oxacillin-resistant (oxacillin MICs, ≥4 μg/ml) strains.

Enterococci included 64 vancomycin-susceptible clinical strains; most of the species were *E. faecalis* or *E. faecium*, and lower numbers were of the species *E. avium*, *E. durans*, *E. hirae*, *E. maleodoratus*, *E. mundii*, *E. pseudoavium*, and *E. raffinosus*. A collection of enterococci selected as belonging to the recognized resistance phenotypes consisted of 33 strains of the VanA phenotype (including 2 laboratory-derived transconjugants [2]), 5 strains of the VanB phenotype, and 20 strains of the VanC phenotype. VanA strains belonged to the species *E. faecium*, *E. faecalis*, *E. avium*, and *E. durans*; VanB strains belonged to the species *E. faecium* and *E. faecalis*; and VanC strains belonged to the species *E. casseliflavus*, *E. flavescens*, and *E. gallinarum.*

Streptococci included 23 clinical isolates of *S. pyogenes*, 33 clinical isolates of *S. pneumoniae*, and lower numbers of the species *S. agalactiae*, *S. bovis*, *S. equi*, *S. mutans*, *S. sanguis*, and *S. thermophilus*. On the basis of the MICs determined by standard broth microdilution tests (26), pneumococci were preliminarily differentiated into penicillin-susceptible (penicillin MICs, <0.1 μg/ml) or penicillin-resistant (penicillin MICs, ≥0.1 μg/ml) strains. Other catalase-negative, vancomycin-susceptible gram-positive cocci included 4 *Gemella*, 4 *Lactococcus*, and 2 *Aerococcus* strains.

Among the listeriae were included 22 wild-type strains and 25 laboratory-derived transconjugants. The former consisted of 16 clinical or food isolates of *L. monocytogenes* (including some previously described strains resistant to particular antibiotics [12]), 3 strains of *L. innocua*, and 1 strain each of *L. ivanovii*, *L. seeligeri*, and *L. welshimeri*. Transconjugants were obtained by conjugative transfer of a *vanA* plasmid under laboratory conditions (Consiglio Nazionale delle Ricerche targeted project PF41.00926); the strains of *L. monocytogenes*, *L. ivanovii*, and *L. welshimeri* received the resistance directly from clinical strains of *E. faecium*, whereas those of *L. innocua* and *L. seeligeri* received the resistance in secondary matings with the *Listeria* transconjugants described above (2). Five transconjugants of each species (all carrying a *vanA* plasmid originally carried by a different wild-type *E. faecium* donor) were tested.

All corynebacteria were multiresistant group JK organisms, and all clostridia were clinical isolates of *Clostridium difficile.*

The lactic acid bacteria inherently resistant to vancomycin included 12 *Lactobacillus* strains, 5 strains of *Leuconostoc mesenteroides*, and 3 strains of *Pediococcus pentosaceus*, all of which were isolated from food or clinical specimens. The strains of *E. rhusiopathiae* were all of animal origin.

All wild-type isolates tested were independent strains freshly isolated in our laboratories or other Italian laboratories; all laboratory-derived strains were obtained and carefully stored in our laboratory. Most isolates were initially identified with commercially available and automated biochemical test systems, but the identifications of several isolates were confirmed by determining additional distinguishing characteristics relevant to the laboratory determination of genus and species.

Susceptibility testing. MICs were determined by standard microdilution procedures, as recommended by the National Committee for Clinical Laboratory Standards (25, 26). Antibiotics were tested at final concentrations (prepared from serial twofold dilutions) ranging from 0.03 to 256 μg/ml for vancomycin and teicoplanin and from 0.03 to 32 μg/ml for LY333328. The MIC was defined as the lowest antibiotic concentration which yielded no visible growth. With aerobic bacteria, the test medium was Mueller-Hinton II broth (BBL Microbiology Systems, Cockeysville, Md.) supplemented with 3% lysed horse blood for strains other than staphylococci and enterococci; the inoculum was 5×10^5 CFU/ml. The inoculated trays were incubated at 35°C for 18 h (in an atmosphere containing 5% CO_2 in the case of most streptococci and JK coryneform bacteria). With *C. difficile* strains, the test medium was brucella broth (Oxoid Ltd., Basingstoke, England) supplemented with hemin and vitamin K_1; the inoculum was 10^6 CFU/ml. The inoculated trays were incubated for 48 h at 35°C in GasPak jars (BBL).

Minimal bactericidal concentrations (MBCs) were established by extending the MIC procedure to the evaluation of bactericidal activity (24). After the MIC was read, 0.01-ml volumes were drawn with an Eppendorf pipette from the wells showing no growth and were spotted onto suitable agar plates. The plates were incubated at 35°C for 24 to 48 h. The MBC was read as the lowest concentration of antibiotic which resulted in ≤0.1% survival in the subculture. Time-kill curve studies were performed in flasks by standard procedures (24). The antibiotics were tested at concentrations 1 to 16 times the MIC. The starting inoculum was 10^5 to 10^6 CFU/ml. Cultures were incubated at 35°C with shaking. At intervals, viable counts were determined by spreading aliquots of 0.1 ml of the suitable dilutions on plates of brain heart infusion agar (Oxoid).

S. aureus ATCC 29213 and ATCC 25923, *E. faecalis* ATCC 29212, and *S. pneumoniae* ATCC 49619 were used as quality control strains.

In vitro selection of resistance. Attempts to select for single-step resistance to LY333328 were performed as described elsewhere (3). An aliquot of 0.1 ml of an overnight broth culture (approximately 10^9 CFU/ml) was spread onto the surface of a plate of brain heart infusion agar containing 10 μg of LY333328 per ml. For each test strain, 40 aliquots were plated onto as many plates. The plates were incubated at 37°C for 48 h and were then examined for colonies denoting surviving clones.

RESULTS AND DISCUSSION

Comprehensive comparisons of the activities (in terms of MICs and MBCs) of LY333328, vancomycin, and teicoplanin are presented in Table 1 (staphylococci), Table 2 (enterococci), Table 3 (streptococci and related organisms, listeriae, corynebacteria, and clostridia), and Table 4 (bacteria of inherently glycopeptide-resistant, gram-positive genera).

MIC tests. Against the *Staphylococcus* strains tested, LY333328 MICs ranged from ≤0.03 to 8 μg/ml and were either identical

TABLE 3. Comparative activities (MICs and MBCs) of LY333328, vancomycin, and teicoplanin against 68 streptococci, 10 strains of other catalase-negative vancomycin-susceptible gram-positive cocci, 47 listeriae, 12 corynebacteria, and 24 strains of *C. difficile*

Organism or organism group (no. of isolates tested)	Antimicrobial agent	MIC (μg/ml)[a]			MBC (μg/ml)[b]		
		Range	50%	90%	Range	50%	90%
Streptococcus pyogenes (23)	LY333328	0.06–0.5	0.125	0.25	0.06–2	0.5	0.5
	Vancomycin	All 0.25	0.25	0.25	0.25–1	0.5	0.5
	Teicoplanin	≤0.03–0.125	≤0.03	0.06	≤0.03–0.25	0.06	0.25
Streptococcus pneumoniae, penicillin-susceptible (21)	LY333328	All ≤0.03	≤0.03	≤0.03	All ≤0.03	≤0.03	≤0.03
	Vancomycin	0.125–1	0.125	0.25	0.125–1	0.25	0.25
	Teicoplanin	≤0.03–0.125	≤0.03	≤0.03	≤0.03–0.25	≤0.03	0.06
Streptococcus pneumoniae, penicillin-resistant (12)	LY333328	All ≤0.03	≤0.03	≤0.03	All ≤0.03	≤0.03	≤0.03
	Vancomycin	0.125–0.25	0.25	0.25	0.125–0.5	0.25	0.25
	Teicoplanin	All ≤0.03	≤0.03	≤0.03	≤0.03–0.06	≤0.03	0.06
Other *Streptococcus* spp. (12)[c]	LY333328	≤0.03–0.5	0.25	0.5	0.06–1	0.5	1
	Vancomycin	0.125–2	0.5	0.5	0.5–8	2	8
	Teicoplanin	≤0.03–0.125	0.06	0.125	0.125–8	1	8
Other catalase-negative vancomycin-susceptible gram-positive cocci (10)[d]	LY333328	All ≤0.03	≤0.03	≤0.03	≤0.03–1	0.125	0.25
	Vancomycin	0.125–0.5	0.125	0.5	0.5–16	4	8
	Teicoplanin	≤0.03–0.125	0.06	0.125	0.25–4	0.5	4
Wild-type *Listeria* strains (22)[e]	LY333328	≤0.03–0.125	≤0.03	0.06	≤0.03–0.5	0.06	0.125
	Vancomycin	0.25–2	0.5	1	1–8	4	8
	Teicoplanin	0.06–0.25	0.125	0.25	0.5–8	2	8
Vancomycin-resistant *Listeria* transconjugants (25)[f]	LY333328	0.5–2	1	2	2–16	4	8
	Vancomycin	All >256	>256	>256	All >256	>256	>256
	Teicoplanin	256–>256	256	>256	All >256	>256	>256
JK coryneform bacteria (12)	LY333328	≤0.03–0.125	0.06	0.125	0.06–0.5	0.25	0.5
	Vancomycin	0.25–0.5	0.25	0.25	0.25–1	0.5	1
	Teicoplanin	0.25–0.5	0.25	0.25	0.25–1	0.5	0.5
Clostridium difficile (24)	LY333328	0.125–2	0.25	1	0.125–4	0.5	2
	Vancomycin	0.5–4	0.5	1	0.5–8	1	4
	Teicoplanin	0.06–0.5	0.125	0.25	0.125–2	0.5	1

[a] See footnote *a* of Table 1.
[b] See footnote *b* of Table 1.
[c] Four strains of *S. bovis*, three strains of *S. agalactiae*, two strains of *S. thermophilus*, and one strain each of *S. equi*, *S. mutans*, and *S. sanguis*.
[d] Four strains of *Gemella morbillorum*, two strains of *Lactococcus lactis* subsp. *lactis*, two strains of *L. lactis* subsp. *cremoris*, and two strains of *Aerococcus aerogenes*.
[e] Thirteen clinical and three food isolates of *L. monocytogenes*, three strains of *L. innocua*, and one strain each of *L. ivanovii*, *L. seeligeri*, and *L. welshimeri*.
[f] Five *vanA*-carrying transconjugants of *L. monocytogenes*, five strains of *L. innocua*, five strains of *L. ivanovii*, five strains of *L. seeligeri*, and five strains of *L. welshimeri*.

to or, more often, twice as high as those of vancomycin. In particular, the activity of LY333328 was comparable to that of vancomycin (MICs, 1 to 8 μg/ml) against the highly teicoplanin-resistant cultures of *S. haemolyticus* and *S. epidermidis*, whether they were clinical or laboratory-derived strains. No significant differences in MICs between oxacillin-susceptible and oxacillin-resistant strains were recorded. The MICs for 90% of the isolates tested (however they were grouped) were consistently 4 μg/ml.

LY333328 was highly active against enterococci, including vancomycin-resistant strains of all three recognized phenotypes, with MICs never exceeding 1 μg/ml. The LY333328 MICs were similar for vancomycin-susceptible and vancomycin-resistant enterococci: the same MICs for 50 and 90% of the isolates tested (0.25 and 0.5 μg/ml, respectively) were recorded for vancomycin-susceptible and VanC isolates, and two times higher MICs were recorded for VanA strains; among the five VanB isolates tested, the MIC for one strain was of 0.5 μg/ml, while the MICs for the remaining four strains were lower. For vancomycin-susceptible enterococci, the MICs of LY333328 for 50 and 90% of the isolates tested were four times lower than those of vancomycin and two times higher than those of teicoplanin.

Pneumococci, both penicillin-susceptible and penicillin-resistant strains, were highly susceptible to LY333328, with MICs being closer to those of teicoplanin than to those (mostly four to eight times higher) of vancomycin. Strains of other *Streptococcus*, *Lactococcus*, *Gemella*, and *Aerococcus* species were very susceptible to LY333328, although for other *Streptococcus* species MICs were slightly higher compared with those for pneumococci.

LY333328 demonstrated potent activity against listeriae, including clinical and food isolates and laboratory-derived transconjugants carrying the *vanA* determinant. Wild-type listeriae were particularly susceptible to the drug, with MICs not exceeding 0.125 μg/ml. For laboratory-derived transconjugants, for which MICs of both vancomycin and teicoplanin were

TABLE 4. Comparative activities (MICs and MBCs) of LY333328, vancomycin, and teicoplanin against 31 strains of inherently vancomycin-resistant gram-positive genera

Organism (no. of isolates tested)	Antimicrobial agent	MIC (μg/ml)[a]			MBC (μg/ml)[b]		
		Range	50%	90%	Range	50%	90%
Lactobacillus spp. (12)[c]	LY333328	4–32	16	32	32–>32	>32	>32
	Vancomycin	All >256	>256	>256	All >256	>256	>256
	Teicoplanin	All >256	>256	>256	All >256	>256	>256
Leuconostoc mesenteroides (5)	LY333328	1–8			8–>32		
	Vancomycin	All >256			All >256		
	Teicoplanin	256–>256			All >256		
Pediococcus pentosaceus (3)	LY333328	2–8			16–>32		
	Vancomycin	All >256			All >256		
	Teicoplanin	All >256			All >256		
Erysipelothrix rhusiopathiae (11)	LY333328	1–4	2	4	8–32	16	32
	Vancomycin	32–64	64	64	256–>256	>256	>256
	Teicoplanin	2–8	2	8	8–32	16	32

[a] See footnote *a* of Table 1.
[b] See footnote *b* of Table 1.
[c] Four strains of *L. bulgaricus*, three strains of *L. acidophilus*, three strains of *L. helveticus*, and two strains of *L. casei*.

≥256 μg/ml, LY333328 MICs were in the range of 0.5 to 2 μg/ml.

JK coryneform bacteria were highly susceptible to LY333328, with MICs not exceeding 0.125 μg/ml and mostly being two to four times lower than those of vancomycin or teicoplanin. For *C. difficile* isolates, LY333328 MICs, ranging from 0.125 to 2 μg/ml, were in most instances slightly lower than those of vancomycin and two to four times higher than those of teicoplanin.

Among the 20 test strains from lactic acid bacteria inherently resistant to vancomycin and teicoplanin (MICs for all strains were ≥256 μg/ml), LY333328 MICs ranged between 1 and 8 μg/ml for *Leuconostoc* and *Pediococcus* isolates and between 4 and 32 μg/ml for *Lactobacillus* isolates; in particular, the only two strains for which the LY333328 MIC was as high as 32 μg/ml belonged to the species *Lactobacillus bulgaricus*. Among the 11 strains of *E. rhusiopathiae*, for all of which vancomycin MICs were 32 or 64 μg/ml, LY333328 MICs were in the range of 1 to 4 μg/ml.

Bactericidal activity. The LY333328 MBCs exceeded the MICs to a relatively variable extent, depending on the organisms. Against staphylococci (irrespective of susceptibility or resistance to teicoplanin or oxacillin), streptococci, listeriae (both wild-type isolates and laboratory-derived strains), corynebacteria, and clostridia, the MBC-to-MIC ratios yielded by LY333328 usually ranged between 1 and 8 and were rather similar, in most instances, to those yielded by vancomycin or teicoplanin. For enterococci, however, the MBC-to-MIC ratios yielded by LY333328 (on average, 4 to 8 for vancomycin-susceptible strains as well as vancomycin-resistant strains) were considerably lower than those yielded by vancomycin or teicoplanin (>16 for most strains). High MBC-to-MIC ratios (>8) were recorded for several isolates of the inherently vancomycin-resistant gram-positive genera.

Three clinical *Enterococcus* isolates (one isolate of the VanA phenotype, one isolate of the VanB phenotype, and one vancomycin-susceptible isolate) and two clinical *Staphylococcus* isolates (one strain of moderately teicoplanin-susceptible *S. aureus* and one strain of teicoplanin-resistant *S. haemolyticus*, both oxacillin resistant) were tested in time-kill assays. Killing curves of LY333328 against the VanA (Fig. 1A) and VanB (Fig. 1B) enterococci revealed 2-log reductions over 24 h at concentrations that were eight times the MICs; 3-log reductions were observed within the first 4 h at concentrations that were 16 times the MICs. Against the vancomycin-susceptible enterococci (Fig. 1C), a 3-log reduction was observed in the first 8 h at an LY333328 concentration that was eight times the MIC. Against the teicoplanin-resistant *Staphylococcus* isolates tested (Fig. 2A and B), the bactericidal activity of LY333328 during the first 4 to 8 h was much faster than that of vancomycin. Unlike vancomycin and teicoplanin, whose killing activities were relatively concentration independent, the killing curves of LY333328 were clearly concentration dependent, particularly for enterococci.

The uniform bactericidal activity of LY333328 against enterococci deserves special attention and may enhance, in perspective, the potential of LY333328 for the treatment of enterococcal infections. In fact, although susceptible on the basis of the results of MIC assays, enterococci are known to be poorly killed by most conventional bactericidal agents (20), including vancomycin, teicoplanin, and other investigational glycopeptides (4).

Attempts to select for resistant clones. Three clinical isolates (one teicoplanin-resistant *S. haemolyticus* isolate [LY333328 MIC, 4 μg/ml], one moderately teicoplanin-susceptible *S. aureus* isolate [LY333328 MIC, 4 μg/ml], and one VanA *E. faecium* isolate [LY333328 MIC, 0.5 μg/ml]) were studied in vitro for their abilities to develop resistance to LY333328. After exposure to the drug, all strains failed to yield resistant survivors that stably grew in the presence of 10 μg of LY333328 per ml.

Conclusion. The most noteworthy features of LY333328 were its activity against gram-positive organisms, both clinical and laboratory-derived strains, resistant to currently available glycopeptides and its uniform bactericidal power against enterococci. The excellent inhibitory and bactericidal activities of LY333328 suggest that it could be a clinically useful alternative for the treatment of severe infections caused by gram-positive pathogens, particularly those resistant or not fully susceptible to the available glycopeptides. Moreover, considering its uniform activity against vancomycin-resistant enterococci, the cur-

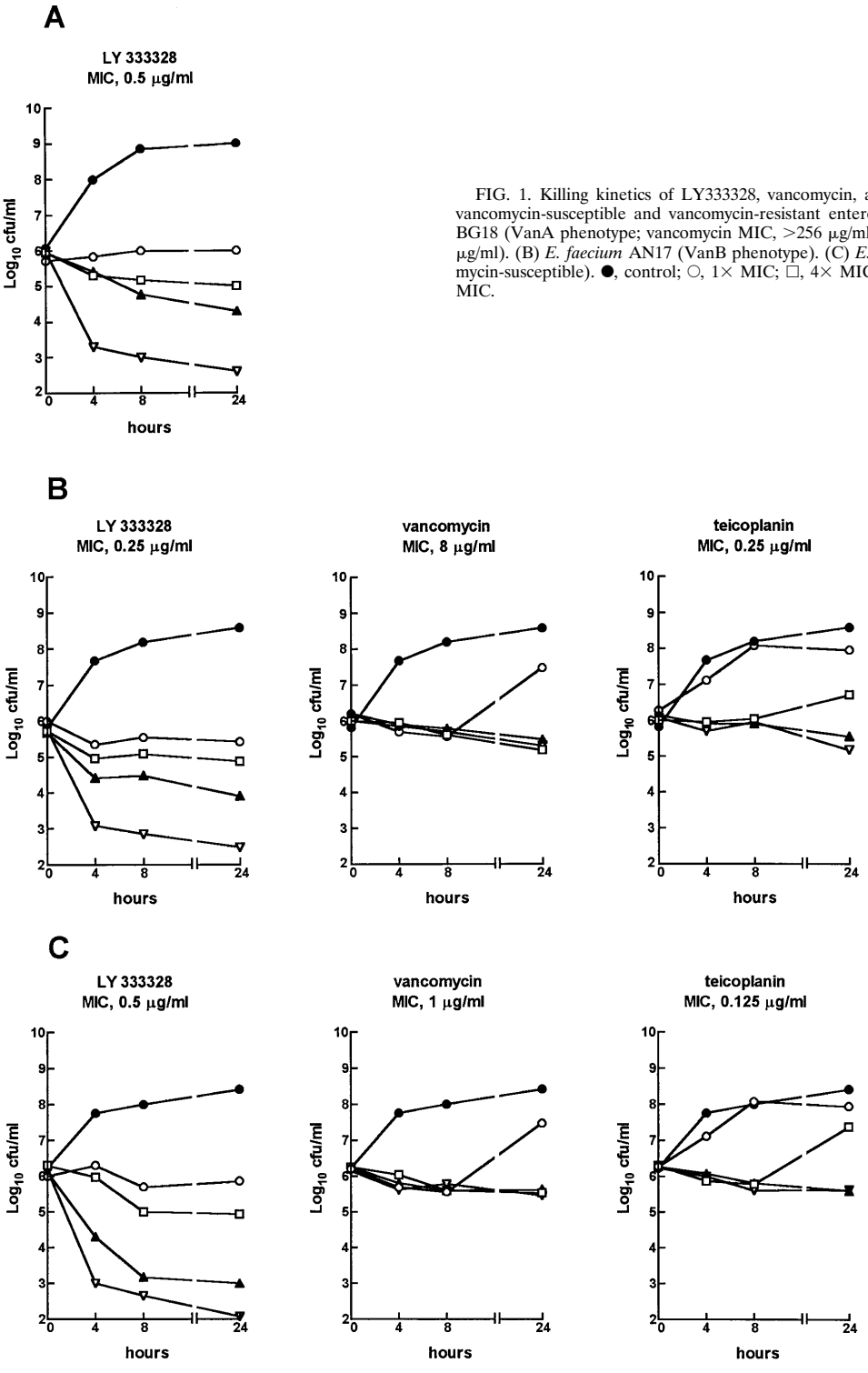

FIG. 1. Killing kinetics of LY333328, vancomycin, and teicoplanin against vancomycin-susceptible and vancomycin-resistant enterococci. (A) *E. faecium* BG18 (VanA phenotype; vancomycin MIC, >256 μg/ml; teicoplanin MIC, 256 μg/ml). (B) *E. faecium* AN17 (VanB phenotype). (C) *E. faecalis* AN91 (vancomycin-susceptible). ●, control; ○, 1× MIC; □, 4× MIC; ▲, 8× MIC; ▽, 16× MIC.

rent recommendations against the use of orally administered glycopeptides for the treatment of *C. difficile*-associated disease or in regimens attempting to decontaminate the intestinal tract (39), which are aimed at preventing the emergence of vancomycin-resistant enterococci in the intestinal flora, are unlikely to apply to LY333328. In preliminary in vivo studies, LY333328 has been reported to have a considerable advantage over vancomycin in terms of its pharmacokinetics, with apparently a much longer half-life in rats (18), and of its efficacy in a mouse protection model against challenge with *S. aureus*, *S. pneumoniae*, or *S. pyogenes* (29) or vancomycin-resistant enterococci (6). In anticipation of clinical trials, further investigations to better elucidate the pharmacological properties and the in vivo efficacy of LY333328 and to

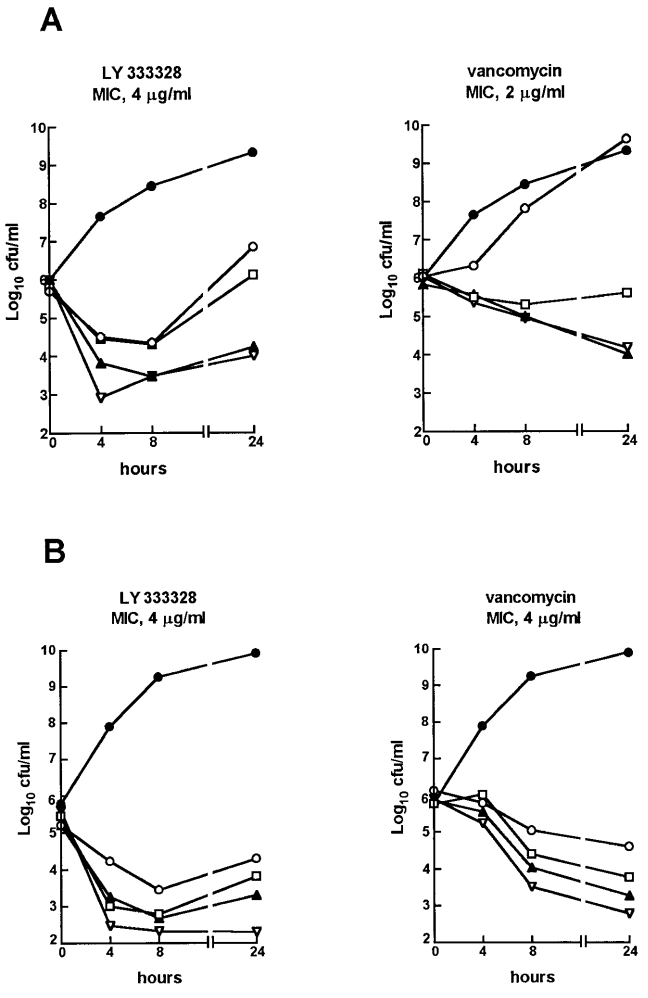

FIG. 2. Killing kinetics of LY333328 and vancomycin against moderately teicoplanin-susceptible and teicoplanin-resistant staphylococci. (A) *S. aureus* CVC4 (moderately susceptible to teicoplanin [MIC, 8 μg/ml], oxacillin resistant). (B) *S. haemolyticus* SH8 (resistant to teicoplanin [MIC, 128 μg/ml], oxacillin resistant). ●, control; ○, 1× MIC; □, 4× MIC; ▲, 8× MIC; ▽, 16× MIC.

evaluate its safety by toxicological studies are warranted and are strongly urged.

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