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Activities of the Semisynthetic Glycopeptide LY191145 against Vancomycin-Resistant Enterococci and Other Gram-Positive Bacteria

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LY191145 is the prototype of a series of compounds with activities against vancomycin-resistant enterococci derived by modification of the glycopeptide antibiotic LY264826. LY191145 had MICs for vancomycin- and teicoplanin-resistant enterococci of ≤4 μg/ml for 50% of isolates and ≤16 μg/ml for 90% of isolates. Its MICs for vancomycin-resistant, teicoplanin-susceptible enterococci were 1 to 8 μg/ml. LY191145 retains the potent activities of its parent compound against staphylococci and streptococci. In vivo studies in a mouse infection model confirmed these activities. This compound indicates the potential of semisynthetic glycopeptides as agents against antibiotic-resistant gram-positive bacteria.

Vancomycin was introduced in 1959 as an alternative treatment for infections by gram-positive bacteria. The emergence of resistance to penicillins, cephalosporins, and other antibiotics has made vancomycin the treatment of choice in many serious infections. Vancomycin is unusual in that resistance did not emerge during its first 30 years of use (4, 6). However, vancomycin resistance is now present in enterococci, and as the genes encoding this resistance are on mobile elements that can move into other bacteria, resistance could spread to staphylococci and streptococci (7, 15). Infections by vancomycin-resistant enterococci are often not treatable by any currently available antibiotic (2, 5), so the need for a new agent is urgent.

Many glycopeptide antibiotics related to vancomycin have been discovered over the last 20 years (22). One of these, LY264826 (A82846B [8–10]), is four to eight times more active than vancomycin (3, 16, 18, 20) but is not useful against vancomycin-resistant bacteria. We have previously reported that modifications of LY264826 and vancomycin by alkylation of the amino sugar can afford highly active agents (8, 11, 12, 14). LY191145, the semisynthetic glycopeptide described here (Fig. 1), is the prototype of a series of compounds with activities against both VanA and VanB enterococcal resistance types as well as potent activities against other gram-positive bacteria.

Antibiotics and antibiotic resistance determinations. Vancomycin and LY264826 were obtained from Eli Lilly & Co. (Indianapolis, Ind.), and teicoplanin was obtained from Gruppo Lepetit (Milan, Italy). LY191145 was prepared as previously described (8). MICs were determined by broth microdilution as recommended by the National Committee for Clinical Laboratory Standards (13) with cation-adjusted Mueller-Hinton broth (Mueller-Hinton II; BBL, Cockeysville, Md.) or Mueller-Hinton II with 5% lysed horse blood (for streptococci). All bacterial isolates were from our collection at Eli Lilly & Co. Enterococci were clinical isolates from the United States and Europe, including isolates from Uttley et al. (19). *Enterococcus faecium* and *Enterococcus faecalis* were the only species of VanA, VanB, and vancomycin-susceptible enterococci represented. VanA and VanB refer to phenotypic designations, with VanA isolates being enterococci resistant to both vancomycin and teicoplanin (MIC, ≥16 μg/ml) and VanB isolates being enterococci resistant to vancomycin (MIC, ≥8 μg/ml) but sus-

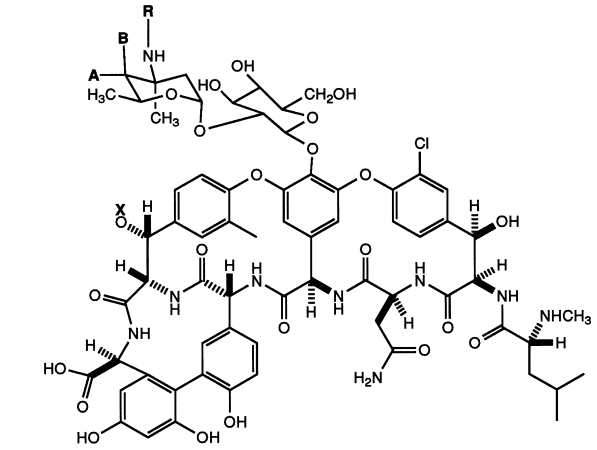

FIG. 1. Structure of the glycopeptide antibiotic LY191145 compared with those of its parent compound (LY264826) and vancomycin.

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TABLE 1. Activities against clinical isolates of enterococci

Enterococci	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
Vancomycin susceptible (27 isolates [14 *E. faecium* and 13 *E. faecalis*])	Vancomycin	1–4	1	2
	Teicoplanin	0.06–2	0.25	1
	LY264826	0.25–1	0.5	0.5
	LY191145	0.13–1	1	1
Vancomycin- and teicoplanin-resistant (VanA) (26 isolates [14 *E. faecium* and 12 *E. faecalis*])	Vancomycin	512–2,048	1,024	1,024
	Teicoplanin	64–512	128	256
	LY264826	4–128	64	256
	LY191145	1–16	4	8
Vancomycin resistant, teicoplanin susceptible (VanB) (20 isolates [10 *E. faecium* and 10 *E. faecalis*])	Vancomycin	32–1,024	256	512
	Teicoplanin	0.5–4	1	2
	LY264826	2–128	16	128
	LY191145	1–8	2	8
E. gallinarum (11 isolates) and *E. casseliflavus* (6 isolates)	Vancomycin	4–16		
	Teicoplanin	0.5–1		
	LY264826	0.25–2		
	LY191145	0.5–1		

[a] 50% and 90%, MICs for 50 and 90% of isolates tested, respectively.

ceptible to teicoplanin (MIC, ≤4 μg/ml). A teicoplanin-resistant isolate derived from a vancomycin-resistant, teicoplanin-susceptible strain (21) was tested separately.

Antibacterial activities in vitro. LY191145 was consistently active against enterococci (Table 1). While LY191145 exhibited potencies similar to those of vancomycin against typical vancomycin-susceptible enterococci, its activities against vancomycin-resistant isolates were superior. *Enterococcus gallinarum* and *Enterococcus casseliflavus* isolates were uniformly susceptible (MICs, ≤1 μg/ml). Most VanB isolates (vancomycin-resistant, teicoplanin-susceptible *E. faecium* and *E. faecalis*) were susceptible to LY191145 (MIC at which 90% of the isolates were inhibited [MIC_{90}], 8 μg/ml). A mutant of a VanB isolate that constitutively expressed resistance to both vancomycin and teicoplanin (21) (MICs of 256 and 64 μg/ml, respectively) was also susceptible to LY191145 (MIC, 4 μg/ml). Against VanA isolates, LY191145 was typically 50 to 200 times more potent than vancomycin. For half of the strains tested the MIC was ≤4 μg/ml. However, for 4 of our 27 isolates the MIC was 16 to 32 μg/ml.

TABLE 2. Activities against clinical isolates of staphylococci

Microorganism	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
Methicillin-resistant *S. aureus* (40 isolates)	Vancomycin	0.5–4	1	2
	Teicoplanin	0.125–8	0.25	1
	LY264826	0.125–2	0.25	1
	LY191145	0.125–2	0.25	0.5
Methicillin-susceptible *S. aureus* (20 isolates)	Vancomycin	1–2	1	2
	Teicoplanin	0.125–1	0.5	0.5
	LY264826	≤0.06–0.5	0.25	0.25
	LY191145	≤0.06–0.5	0.25	0.25
Methicillin-resistant *Staphylococcus epidermidis* (30 isolates)	Vancomycin	1–4	2	4
	Teicoplanin	1–32	2	16
	LY264826	0.5–4	2	2
	LY191145	0.25–4	1	2

[a] See Table 1, footnote *a*.

TABLE 3. Activities of LY191145 and other glycopeptide antibiotics against coagulase-negative staphylococci that are teicoplanin insensitive

Species and strain	MIC (μg/ml)			
	Vancomycin	Teicoplanin	LY 264826	LY 191145
Staphylococcus haemolyticus ST105	4	64	2	8
Staphylococcus haemolyticus ST065	2	8	0.5	2
Staphylococcus haemolyticus ST339	2	2	0.5	2
Staphylococcus saprophyticus ST338	2	16	2	2
Staphylococcus saprophyticus ST435	0.5	8	0.5	2
Staphylococcus saprophyticus ST436	2	8	0.5	1
Staphylococcus warneri ST362	1	4	0.5	0.25

We noted that many of our clinical isolates of *E. faecium* and *E. faecalis* grew quite poorly in Mueller-Hinton medium and had to exclude some strains that did not grow well enough to prepare test inocula. We also tested our compounds in brain heart infusion broth, which allowed uniform growth of all the enterococci examined. The activities of all the glycopeptides tested in brain heart infusion broth were similar, yielding the same MIC_{90}s for vancomycin- and teicoplanin-resistant isolates and MIC_{90}s and MIC_{50}s of experimental compounds that were two- to fourfold lower against vancomycin-susceptible isolates (data not shown).

LY191145 was also consistently active against staphylococci (Tables 2 and 3). The parent compound of LY191145, LY264826, is more active than vancomycin against *Staphylococcus aureus*, and this superior potency is retained by LY191145. LY191145 was also more active than both vancomycin and teicoplanin against typical strains of *Staphylococcus epidermidis* and more active than teicoplanin against most teicoplanin-resistant isolates of coagulase-negative staphylococci. Like other glycopeptide antibiotics, LY191145 showed uniform activities against both penicillin-susceptible and penicillin-resistant pneumococci but was 8 to 32 times more potent than vancomycin (MIC, ≤0.03 μg/ml) (Table 4).

Antibacterial efficacies in vivo. A standard mouse protection test was carried out as previously described (11). Mice were infected by interperitoneal administrations of bacteria. *S. aureus* was administered in a suspension containing 5% hog gastric mucin (Sigma, St. Louis, Mo.). Antibiotics were administered by subcutaneous injections 1 and 5 h after infection. Deaths were recorded over 7 days, and the 50% effective dose was calculated by the method of Reed and Muench (17). LY191145 was more active than vancomycin against *Strepto-*

TABLE 4. Activities against streptococci

Species and strain	MIC (μg/ml)				
	Penicillin G	Vancomycin	Teicoplanin	LY 264826	LY 191145
Streptococcus pyogenes C-203	0.008	0.25	0.06	0.06	0.016
Streptococcus pneumoniae Park I	0.016	0.5	0.13	0.13	0.03
Streptococcus pneumoniae SP325	0.06	0.5	0.13	0.13	0.06
Streptococcus pneumoniae SP328	8	0.25	0.13	0.13	0.016
Streptococcus pneumoniae SP329	4	0.25	0.13	0.06	0.016
Streptococcus pneumoniae SP330	2	0.25	0.13	0.06	0.008
Streptococcus pneumoniae SP331	1	0.25	0.13	0.06	0.016
Streptococcus pneumoniae SP376	2	0.25	0.06	0.06	0.016
Streptococcus pneumoniae SP377	4	0.25	0.13	0.06	0.016
Streptococcus pneumoniae SP3770	2	0.25	0.06	0.06	0.008

TABLE 5. Activities of LY191145 and related glycopeptides in a mouse protection model

Antimicrobial agent	ED_{50} (mg/kg) for[a]:		
	Streptococcus pneumoniae Park I	*Streptococcus pyogenes* C-203	*S. aureus* 3055b
Vancomycin	1.1	0.80	1.2
LY264826	0.18	0.18	0.20
LY191145	0.087	0.062	0.25

[a] ED_{50}, median effective dose. Infection was established by interperitoneal bacterial challenge; treatment was two subcutaneous doses 1 and 5 h after challenge.

coccus pneumoniae, *Streptococcus pyogenes*, and *S. aureus* (Table 5).

Rat pharmacokinetic properties. The plasma pharmacokinetics of LY191145 were assessed in male Sprague-Dawley rats (three per time point) administered a 5-mg/kg dose via the tail vein; blood samples were collected periodically over 24 h. After solid-phase extraction of plasma, glycopeptide was quantitated by high-pressure liquid chromatography with UV detection. Plasma protein binding was measured at 10 and 50 μg/ml by standard ultrafiltration or ultracentrifugation techniques.

The terminal-phase half-life of LY191145 in plasma was approximately three times greater than that measured for vancomycin (Table 6) and was similar to that reported for teicoplanin (Table 6) (1). Plasma protein binding was independent of the concentration for all of the glycopeptides and concentrations tested. When corrected for protein binding, the plasma clearance of LY191145 was similar to those of vancomycin and teicoplanin. The steady-state volume of distribution of LY191145, corrected for protein binding, was 3.8 times greater than that of vancomycin. Both the larger volume of distribution and higher plasma protein binding probably contribute to the greater half-life of LY191145 compared with that of vancomycin.

Summary and conclusions. LY191145 illustrates the potential of alkyl-modified glycopeptides as agents against resistant gram-positive bacteria. This compound exhibits in vitro activities against *S. aureus* and other staphylococci, including methicillin-resistant *S. aureus* and teicoplanin-resistant staphylococci, and shows potent in vitro activities against streptococci, including penicillin-resistant *S. pneumoniae*. The results of initial efficacy testing in a mouse protection model confirmed these activities. Pharmacokinetic studies showed favorable properties, including a longer half-life and moderate plasma protein binding. Most interesting, however, are the activities of LY191145 against enterococci, especially vancomycin-resistant *E. faecium* and *E. faecalis*. While the activities of this compound may be inadequate to anticipate clinical efficacy, LY191145 shows that this type of modification of highly active glycopeptide antibiotics can yield interesting agents. We plan to explore further the activities and potential of N-alkyl-substituted glycopeptides.

TABLE 6. Rat pharmacokinetic properties of LY191145 and other glycopeptides

Antimicrobial agent	Half-life (h)	Clearance (liter/h/kg)[a]	Vol. of distribution at steady state (liter/kg)[a]	Plasma protein binding (%)
Vancomycin	0.85	0.39 (0.68)	0.33 (0.58)	43
LY264826	1.1	0.18 (0.30)	0.18 (0.30)	39
Teicoplanin	2.2	0.08 (0.73)	0.22 (2.0)	89
LY191145	2.7	0.20 (0.74)	0.59 (2.2)	73

[a] Numbers in parentheses are values corrected for protein binding.

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