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Antimicrobial Activity of MDL 63,246, a New Semisynthetic Glycopeptide Antibiotic

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MDL 63,246 is a semisynthetic derivative of the naturally occurring glycopeptide antibiotic MDL 62,476 (A40926). It was more active in vitro against *Staphylococcus aureus* and coagulase-negative staphylococci than MDL 62,476, teicoplanin, and vancomycin and was more active than mideplanin (MDL 62,873) against some isolates. MDL 63,246 had excellent activity against streptococci and teicoplanin-susceptible enterococci, and it also had in vitro activity against some VanA enterococcal isolates. It was more active than teicoplanin and vancomycin against acute staphylococcal, streptococcal, and enterococcal septicemia in immunocompetent and neutropenic mice. It was highly efficacious in reducing the bacterial load in the hearts of rats in staphylococcal endocarditis experiments and the bacterial load of *Staphylococcus epidermidis* in a thigh infection model in neutropenic mice. The excellent in vivo activity of MDL 63,246 appears to correlate both with its in vitro antibacterial activity and with its long half-life in rodents.

Glycopeptide antibiotics are an important part of the armamentarium against gram-positive infections, particularly those caused by enterococci and methicillin-resistant staphylococci. Acquired high-level resistance to glycopeptides in enterococci is an emerging problem (8, 13, 16, 17, 23, 25), and intermediate susceptibility or resistance to teicoplanin, and occasionally to vancomycin, occurs in methicillin-resistant coagulase-negative staphylococci (1, 12, 14, 20, 24).

Some amide derivatives of teicoplanin, such as mideplanin (MDL 62,873), have been demonstrated to have better activity than teicoplanin against coagulase-negative staphylococci (2–4, 14). We have recently turned our attention to chemical derivatives of MDL 62,476 (A40926), a glycopeptide antibiotic whose antibacterial activity is similar to that of teicoplanin except for its additional activity against *Neisseria gonorrhoeae* (11). We present data on the in vitro activity and efficacy in animal infection models of a new amide derivative of this antibiotic, MDL 63,246 (Fig. 1).

MATERIALS AND METHODS

Antimicrobial agents. Teicoplanin, mideplanin (MDL 62,873), MDL 62,476 (A40926), and MDL 63,246 were provided by the Lepetit Research Center. The structures of these compounds are given in Fig. 1. Vancomycin, tetracycline, penicillin G, clindamycin, and erythromycin were obtained from Sigma Chemical Co. (St. Louis, Mo.).

MIC determination. Isolates were obtained from various clinical sources. The set of staphylococci used for these experiments was biased to include a high proportion of strains for which teicoplanin MICs were known to be high. The three *Staphylococcus aureus* isolates for which teicoplanin MICs were 8 to 16 μg/ml were kindly provided by J. Acar, G. Kaatz, and G. C. Schito. The enterococci included vancomycin-resistant isolates belonging to various phenotypic classes. MIC determinations were performed by the broth microdilution methodology with inocula of approximately 5×10^5 CFU/ml (18). The media used included Iso-Sensitest broth (Oxoid) and cation-adjusted Mueller-Hinton broth (Difco) for staphylococci; cation-adjusted Mueller-Hinton broth for enterococci, bacilli, and micrococci; Mueller-Hinton broth supplemented additionally with 5% (vol/vol) fetal calf serum for streptococci or with 5% lysed horse blood for listeria; MRS broth (Difco) for pediococci, lactobacilli, lactococci, and leuconostocs; brain heart infusion broth (Difco) plus 10% (vol/vol) fetal calf serum for corynebacteria; and GC base broth (Oxoid) supplemented with 1% (vol/vol) IsoVitaleX (BBL) for *N. gonorrhoeae*. *N. gonorrhoeae* was incubated for 48 h at 37°C in 5% CO_2; all other organisms were incubated for 24 h at 37°C in air. For all MIC determinations, a small amount of protein (final concentration of bovine serum albumin, 0.01% [wt/vol], fraction V; Sigma) was added to the diluent, because we found that MDL 63,246, like ramoplanin (21), tends to adsorb to plastic surfaces. This supplement was found to have no effect on the activity of the comparison agents.

Bactericidal activity. Several colonies from overnight growth on Mueller-Hinton agar (Difco) were inoculated into cation-adjusted Mueller-Hinton broth (Difco) or Mueller-Hinton II broth (BBL; cation adjusted by the manufacturer) and were incubated with moderate agitation at 37°C. After two or more generations of logarithmic growth, the cultures were diluted to 10^5 to 10^6 CFU/ml with fresh prewarmed broth, and 10-ml aliquots were added to prewarmed flasks with or without the addition of antibiotics. The antibiotic concentrations chosen were four- and eightfold the lowest concentration that inhibited visible growth for at least 24 h in preliminary experiments in flasks. The flasks were agitated as described before, and samples were obtained at intervals up to 48 h. Duplicate 0.1-ml aliquots of suitable dilutions (at least 10-fold) in 0.9% NaCl–0.1% Difco peptone (PSS) were plated by inclusion (in 2.5 ml of Mueller-Hinton broth containing 0.7% agar) on Mueller-Hinton agar plates (Difco). No carryover effects were detected by this method in preliminary experiments in which low inocula of the isolates (10^2 to 10^3 CFU/ml) were plated immediately after the addition of the same concentrations of the antibacterial agents used in these experiments. Colonies were counted after 24 h of incubation at 37°C.

Induction of neutropenia in mice. Female NMRI mice (ages, 6 to 8 weeks; Iffa-Credo) were treated intraperitoneally with 150 mg of cyclophosphamide (Endoxan; Asta) per kg of body weight on days 5, 3, and 1 before infection. The animals were housed throughout the experiments in a cabinet ventilated with air filtered with a HEPA filter. In each experiment, a group of five animals was reserved for monitoring neutropenia. Blood was collected from the tail vein just before the first cyclophosphamide treatment and 24 h after the last treatment. Total leukocytes were determined either with an automated Coulter counter (Ultralogic 400, Clay Adams) or manually with a counting chamber. Differential count was determined by microscopic examination after staining with May-Grünwald-Giemsa stain. In the experiments discussed in this report, the leukocyte counts fell from a pretreatment mean of $13.4 \times 10^3/mm^3$ to a mean of $1.5 \times 10^3/mm^3$ at the time that the other groups of animals were infected (24 h after the third cyclophosphamide treatment). At that time, only lymphocytes were present, whereas prior to cyclophosphamide treatment, lymphocytes, neutrophils, eosinophils, and monocytes represented approximately 90, 10, 0.2, and 0.2% of the population, respectively. Previous studies indicated that

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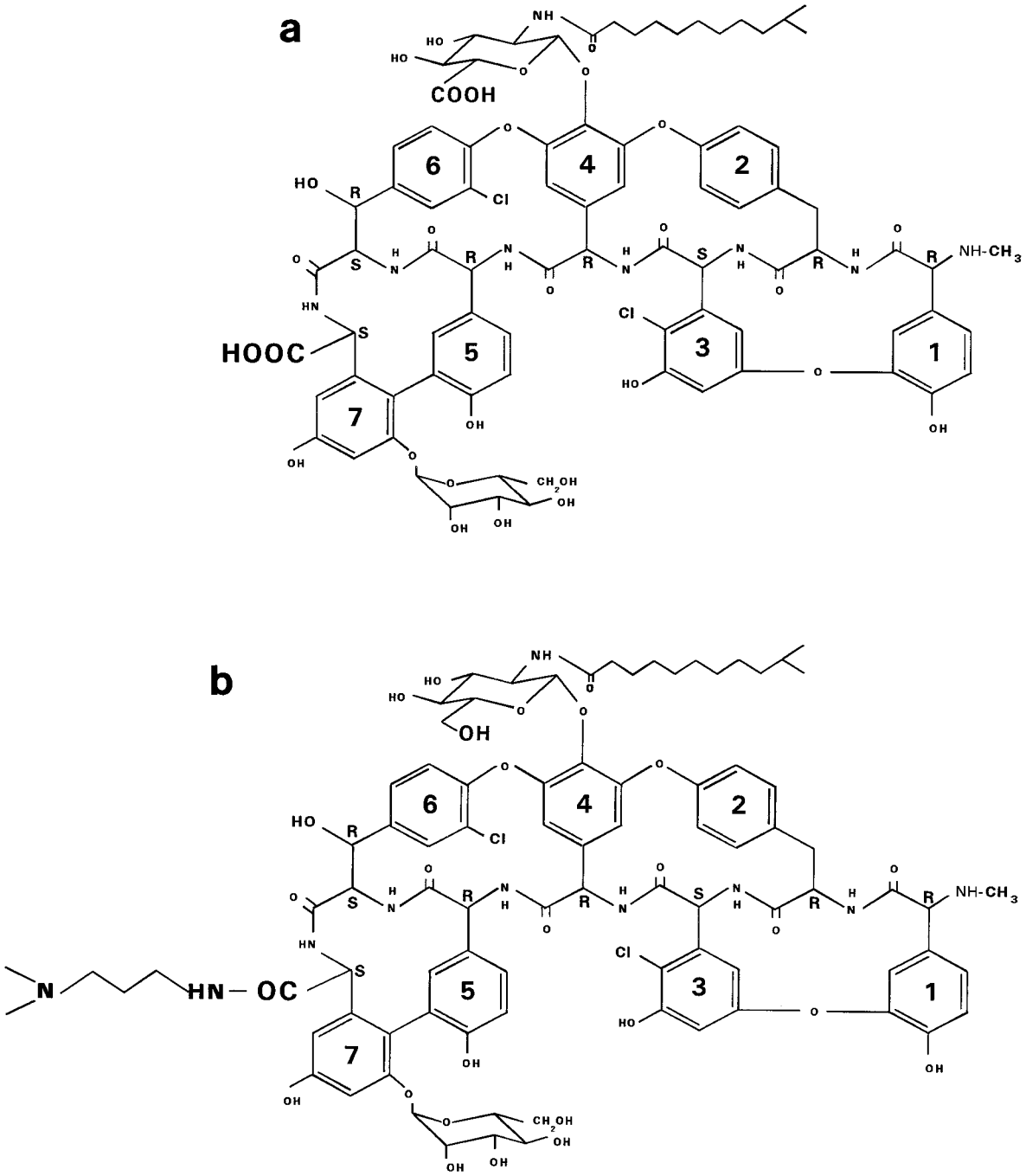

FIG. 1—*Continued on following page.*

neutropenia persists for at least 3 days after the end of cyclophosphamide treatment.

Prophylaxis of septicemia in mice. Acute septicemia experiments in immunocompetent CD1 mice (six animals per treatment group; Charles River) were performed as described previously (3). The bacterial inocula (administered intraperitoneally) which ranged from 70 to 150 times the 50% lethal doses for untreated animals, were 1.6×10^6, 1.2×10^2, and 1.9×10^2 for *S. aureus* Smith, *Streptococcus pyogenes* L49, and *Streptococcus pneumoniae* L44, respectively. Treatment with antibiotics was by the subcutaneous route and was begun within 10 min after infection; in the animals with streptococcal infections, penicillin G and vancomycin were administered a second time, at 5 h after infection.

Staphylococcus epidermidis L1480 and *Enterococcus faecalis* L1139 were grown overnight with agitation at 37°C in brain heart infusion broth (Difco) and were diluted in 5% Difco bacteriological mucin. Neutropenic mice were inoculated intraperitoneally with 0.5 ml of a bacterial suspension containing 7×10^4 CFU of *S. epidermidis* or 7×10^3 CFU of *E. faecalis* (approximately 3,000 times the 50% lethal doses of these isolates). With these inocula, all untreated animals died within 72 h; in immunocompetent animals, much higher inocula of these isolates (10^8 CFU per mouse) are required to obtain this result. For each antibiotic tested, five groups of five mice each were treated subcutaneously with different dosages administered once within 10 min after infection. Vancomycin dosages were administered a second time, at 5 h after infection. The 50% effective doses and 95% confidence limits were calculated by the Spearman-Kärber method (9) from the percentages of animals receiving each dose surviving to day 7. Additional groups of five untreated animals, inoculated with serial 10-fold dilutions of the bacterial suspension, were used to determine the lethal inoculum (50% lethal dose) of the strains by using the same statistical method used for the 50% effective dose.

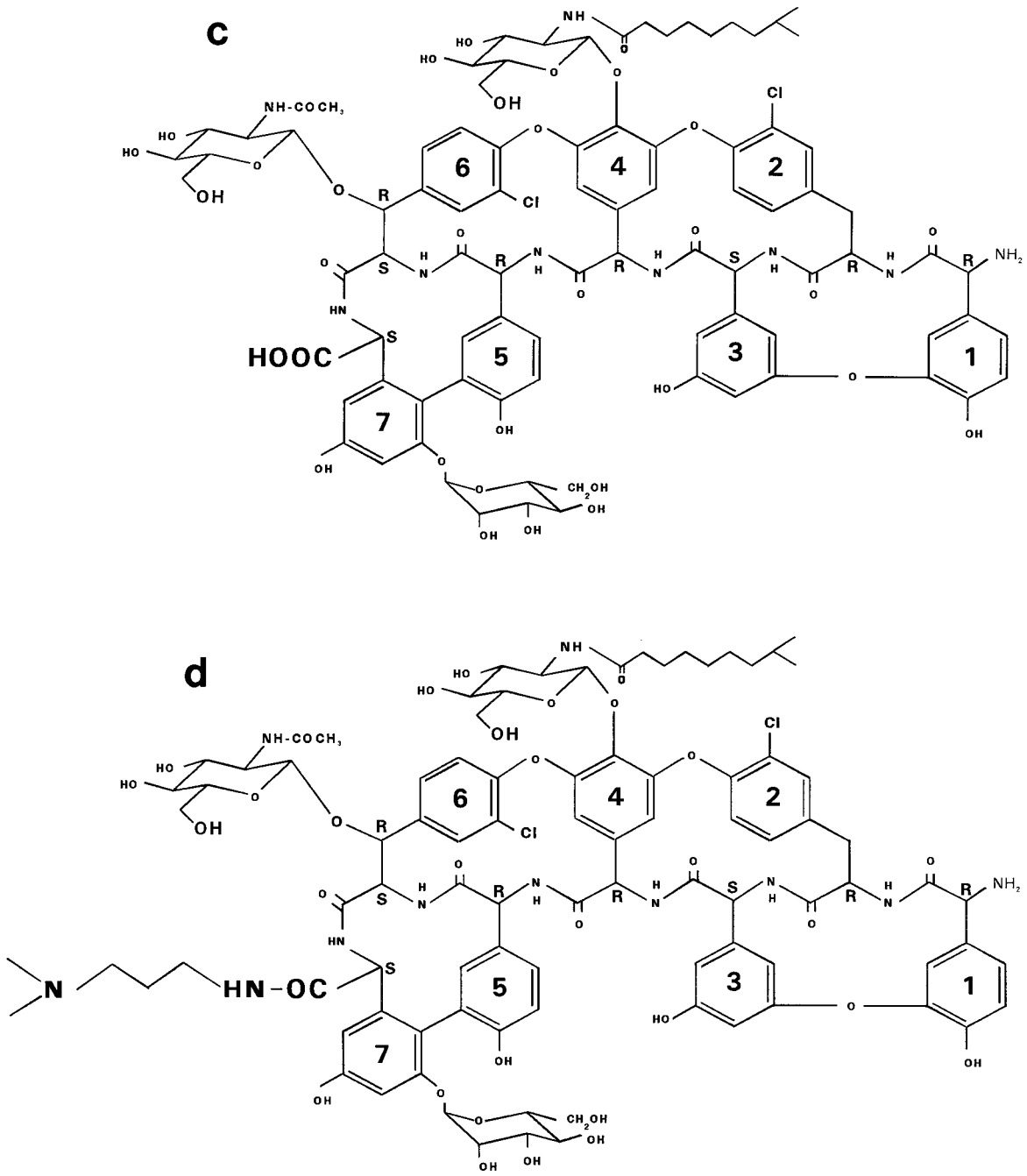

FIG. 1. Chemical structures of MDL 62,476 (a), MDL 63,246 (b), teicoplanin (c) and mideplanin (d).

Thigh infection in neutropenic mice. *S. epidermidis* L1480 was grown to an optical density at 580 nm of 0.1 on a rotary shaker in Difco Todd-Hewitt broth, and the left thigh of each animal was inoculated with 10^5 to 10^6 CFU diluted in 0.1 ml of broth. In one experiment, antimicrobial agents were administered intravenously 2 h after infection, and groups of three to five animals each were killed at intervals up to 24 h, starting just before antibiotic administration. In the second experiment, the comparison agents (teicoplanin and vancomycin) were administered twice, at 2 and 6 h after infection, and groups of animals were killed at intervals up to 48 h. The infected thigh muscles were removed, homogenized in 10 ml of PSS, and filtered through sterile gauze. One-milliliter portions of the filtered homogenates were centrifuged at 12,250 × *g* for 10 min at room temperature, and the pellets were resuspended in 1 ml of PSS. Duplicate 0.05-ml samples of suitable dilutions were spread onto Todd-Hewitt agar plates, which were incubated at 37°C for 24 h. Analysis of variance was performed on the values of $\log_{10}$ CFU per thigh; $P < 0.05$ was considered significant.

Endocarditis in rats. Endocarditis experiments were performed as described previously (3) with two isolates of *S. aureus* and one of *S. epidermidis*. For one of the *S. aureus* isolates the teicoplanin MIC was 8 μg/ml, and for the *S. epidermidis* isolate the teicoplanin MIC was 16 μg/ml. Briefly, a polyethylene catheter was inserted through the aortic valve into the left ventricle via the right carotid artery and was left in place throughout the experiment. Two days later, the animals were infected intravenously. The inocula were 2.6×10^4, 1.5×10^5, and 6.4×10^8 CFU per rat for *S. aureus* L1524, *S. aureus* L561, and *S. epidermidis* L537, respectively. Antimicrobial agents were administered intravenously for 5 days starting 17 h after *S. aureus* infection and 20 h after *S. epidermidis* infection. In order to compensate for the different pharmacokinetics of the three glycopeptides, MDL 63,246 was administered every 24 h at doses of 10 or 20 mg/kg

TABLE 1. Activity of MDL 63,246 against staphylococci[a]

Species	Antimicrobial agent	Iso-Sensitest broth				Mueller-Hinton broth			
		No. of isolates	MIC (μg/ml)			No. of isolates	MIC (μg/ml)		
			Range	50%	90%		Range	50%	90%
S. aureus	MDL 63,246	10	0.06–0.25	0.13	0.13	10	0.06–0.5	0.06	0.25
	MDL 62,476		0.13–8	0.25	4				
	Teicoplanin		0.13–8	0.5	8		0.5–16	0.5	8
	Vancomycin		1–4	1	2				
	Mideplanin		0.13–2	0.25	1				
S. epidermidis	MDL 63,246	10	0.06–1	0.13	0.5	10	0.016–0.13	0.06	0.13
	MDL 62,476		1–16	8	16				
	Teicoplanin		0.5–16	8	16		0.5–32	4	16
	Vancomycin		1–8	2	8				
	Mideplanin		0.06–2	0.13	1				
S. haemolyticus	MDL 63,246	14	0.13–2	0.13	1	10	0.06–0.13	0.06	0.13
	MDL 62,476		2–128	16	64				
	Teicoplanin		1–64	16	32		4–64	16	32
	Vancomycin		1–4	2	4				
	Mideplanin		0.13–4	0.5	2				
Other species	MDL 63,246	6[b]	0.06–1	0.13		10[c]	0.016–0.06	0.03	0.03
	MDL 62,476		0.25–16	2					
	Teicoplanin		0.13–8	1			0.13–4	0.13	0.5
	Vancomycin		1–2	2					
	Mideplanin		0.13–0.5	0.13					

[a] The set of staphylococcal isolates was biased to include a number of isolates for which teicoplanin MICs were known to be relatively high.
[b] One isolate each of *S. cohnii*, *S. lugdunensis*, *S. hominis*, *S. gallinarum*, *S. saprophyticus*, and *S. schleiferi.*
[c] One isolate each of *S. capitis*, *S. cohnii*, *S. hominis*, *S. lugdunensis*, *S. saprophyticus*, *S. sciuri*, *S. schleiferi*, *S. simulans*, *S. warneri*, and *S. xylosus.*

(loading dose, 20 or 40 mg/kg) and teicoplanin was administered every 12 h at 20 mg/kg (loading dose, 40 mg/kg). The surviving animals were killed on day 7 after infection (24 h after the fifth dose of MDL 63,246; 12 h after the 10th dose of teicoplanin). The hearts of all animals were homogenized and processed to determine the bacterial loads.

Bacterial titers were examined by analysis of variance by Scheffe's test for multiple comparisons (19). The mean ± standard error survival time was calculated by survival analysis by the SAS Lifetest procedure (19); differences in survival times between control and treatment groups were evaluated by analysis of variance of the reciprocals of the survival times. Differences in the numbers of survivors and the numbers of sterile vegetations between controls and treated groups were analyzed by the chi-square test (19). In survival time and chi-square analyses, P was corrected for multiple comparisons (P') by the formula $P' = 1 - (1 - P)^n$, where n is the number of preplanned comparisons.

MDL 63,246 and teicoplanin concentrations in plasma were determined by a microbiological assay with *Micrococcus luteus* ATCC 9341 as the test organism.

RESULTS

In vitro activity of MDL 63,246. The activity of MDL 63,246 against staphylococci was tested in two experiments with different media (Table 1). The teicoplanin MIC ranges and the teicoplanin MICs that inhibited 50% (MIC_{50}s) and 90% (MIC_{90}s) of isolates tested were similar in the two experiments, although MDL 63,246 appeared to be somewhat more active against coagulase-negative isolates in Mueller-Hinton broth than in Iso-Sensitest broth. In either medium, MDL 63,246 was more active than teicoplanin; this was particularly evident because the set of staphylococcal isolates was biased to include a high proportion of coagulase-negative staphylococci that were resistant to or of intermediate susceptibility to teicoplanin and three *S. aureus* strains for which teicoplanin MICs were 8 to 16 μg/ml. As reported previously (22), the parent compound of MDL 63,246 (MDL 62,476) had activity similar to that of teicoplanin. MDL 63,246 was more active than vancomycin against staphylococci and was even somewhat more active than mideplanin, a semisynthetic derivative of teicoplanin whose increased activity against coagulase-negative staphylococci has been previously reported (2, 3, 14).

MDL 63,246, MDL 62,476, and teicoplanin had similar activities against vancomycin-susceptible enterococci (Table 2). MDL 63,246 was as active as teicoplanin against phenotypically VanB and VanC isolates and also had moderate activity against the majority of the VanA (teicoplanin-resistant) isolates tested. Intrinsically glycopeptide-resistant species of *Pediococcus*, *Lactobacillus*, and *Leuconostoc* were, however, resistant to MDL 63,246 (Table 3). MDL 63,246 was somewhat more active than teicoplanin against isolates of *S. pyogenes* and *Corynebacterium* spp., but the two compounds had similar activities against other species of streptococci, *Listeria monocytogenes*, and other gram-positive isolates (Table 3).

As already observed for the parent compound (11), MDL 63,246 had activity against *N. gonorrhoeae*, including penicillinase-producing and chromosomally determined penicillin-resistant isolates (Table 4).

Figure 2 shows the results of time-kill experiments performed with three isolates of *S. aureus*, including one (isolate L1524) used in an experimental endocarditis experiment. MDL 63,246 demonstrated slow, time-dependent bactericidal activity, as is typically observed with glycopeptide antibiotics (6, 7, 10). As expected from its relatively low MICs, MDL 63,246 had significant bactericidal activity (at least 99.9% killing) against all three isolates after 48 h of exposure to 4 μg/ml. The extent of killing was generally lower at 24 h or with 2 μg of MDL 63,246 per ml. Vancomycin at 16 μg/ml performed as well as 4 μg of MDL 63,246 per ml against *S. aureus* L1524 and L165 (Fig. 2a and b), but it gave a somewhat lower level of killing at 48 h against L1658 (Fig. 2c), although its initial rate of killing seemed to be greater than that of MDL 63,246

TABLE 2. Activity of MDL 63,246 against enterococci

Species (no. of isolates)	Antimicrobial agent	MIC (μg/ml)		
		Range	50%	90%
E. faecalis (11)	MDL 63,246	0.06–0.13	0.13	0.13
	MDL 62,476	0.13–1	0.13	0.25
	Teicoplanin	0.13–0.5	0.13	0.25
	Vancomycin	1–2	1	2
E. faecium (7)	MDL 63,246	0.03–0.13	0.06	
	MDL 62,476	0.06–0.25	0.13	
	Teicoplanin	0.06–0.25	0.13	
	Vancomycin	0.5–8	1	
E. durans (7)	MDL 63,246	0.06–0.5	0.13	
	MDL 62,476	0.13–0.25	0.25	
	Teicoplanin	0.13–0.25	0.13	
	Vancomycin	0.5–1	1	
Enterococcus spp. (2)[a]	MDL 63,246	0.03–0.06		
	MDL 62,476	0.06–0.13		
	Teicoplanin	0.03–0.06		
	Vancomycin	0.25–0.5		
VanA (19)[b]	MDL 63,246	4–64	16	32
	Teicoplanin	64–>128	>128	>128
	Vancomycin	>128	>128	>128
VanB (7)[c]	MDL 63,246	0.016–0.13	0.06	
	Teicoplanin	0.13–0.5	0.13	
	Vancomycin	64–>128	128	
VanC (6)[d]	MDL 63,246	0.03–0.13	0.06	
	Teicoplanin	0.13–1	0.25	
	Vancomycin	4–8	8	

[a] One isolate each of *E. hirae* and *E. saccharolyticus.*
[b] Eleven *E. faecium* and eight *E. faecalis* isolates.
[c] Four *E. faecium* and three *E. faecalis* isolates.
[d] Four *E. gallinarum* and two *E. casseliflavus* isolates.

against this isolate. The bactericidal activity of 16 μg of teicoplanin per ml was equivalent to or slightly better than that of 4 μg of MDL 63,246 per ml.

Activity of MDL 63,246 against septicemia in mice. On the basis of the dosages of MDL 63,246 which protected 50% of infected animals, MDL 63,246 was more active than teicoplanin against *S. aureus* and *S. pyogenes* septicemia in immunocompetent mice, whereas the two compounds had similar activities against *S. pneumoniae* (Table 5). A single administration of MDL 63,246 was more active than two administrations of penicillin G against the streptococcal infections. Vancomycin was less active than MDL 63,246 and teicoplanin in these experiments, even when it was administered twice.

In neutropenic mice infected with *S. epidermidisi*, MDL 63,246 was more efficacious than teicoplanin and was more effective than two administrations of vancomycin (Table 6). Against a vancomycin-susceptible *E. faecalis* isolate, MDL 63,246 and teicoplanin had similar activities, while vancomycin was less active (Table 6).

Activity of MDL 63,246 against thigh infection in neutropenic mice. The results of experiments measuring the activity of MDL 63,246 against thigh infection in neutropenic mice are given in Fig. 3. In a 24-h experiment (Fig. 3a), MDL 63,246 administration produced a dose-dependent reduction in the bacterial load, with about a 1-log greater reduction at 24 h after treatment with 20 mg/kg than with 5 mg/kg. At either dose, the bacterial titers were significantly lower than those in

TABLE 3. Activity of MDL 63,246 against other gram-positive species

Species (no. of isolates)	Antimicrobial agent	MIC (μg/ml)		
		Range	50%	90%
S. pyogenes (16)	MDL 63,246	0.002–0.03	0.008	0.008
	MDL 62,476	0.016–0.06	0.06	0.06
	Teicoplanin	0.016–0.06	0.03	0.03
S. pneumoniae (17)	MDL 63,246	0.008–0.03	0.016	0.03
	MDL 62,476	0.016–0.06	0.03	0.06
	Teicoplanin	0.03–0.06	0.03	0.06
Streptococcus spp. (23)[a]	MDL 63,246	0.008–0.13	0.03	0.06
	MDL 62,476	0.06–1	0.13	0.5
	Teicoplanin	0.016–0.25	0.06	0.13
Corynebacterium spp. (12)[b]	MDL 63,246	0.13–0.25	0.13	0.13
	Teicoplanin	0.13–1	0.5	1
	Vancomycin	0.5–1	1	1
L. monocytogenes (14)	MDL 63,246	0.06		
	Teicoplanin	0.13		
	Vancomycin	0.5–1	0.5	1
Bacillus spp. (8)	MDL 63,246	0.008–0.5	0.06	
	Teicoplanin	0.03–1	0.06	
	Vancomycin	0.13–1	0.25	
Micrococcus spp. (8)	MDL 63,246	0.016–0.06	0.03	
	Teicoplanin	0.03–0.13	0.06	
	Vancomycin	0.06–0.5	0.25	
Lactococcus garviae (1)	MDL 63,246	0.25		
	Teicoplanin	0.5		
	Vancomycin	1		
Pediococcus spp. (8)[c]	MDL 63,246	16–>128	128	
	MDL 62,476	128–>128	>128	
	Teicoplanin	64–>128	>128	
	Penicillin G	0.25–2	0.5	
	Erythromycin	0.5–2	1	
	Clindamycin	0.06–16	0.13	
Lactobacillus spp. (3)[d]	MDL 63,246	0.03–0.13		
	MDL 62,476	0.13–0.5		
	Teicoplanin	0.13		
	Penicillin G	0.03–0.06		
	Erythromycin	0.13–0.25		
	Clindamycin	0.06–16		
Lactobacillus spp. (4)[e]	MDL 63,246	32–>128		
	MDL 62,476	>128		
	Teicoplanin	>128		
	Penicillin G	0.13–32		
	Erythromycin	0.25–1		
	Clindamycin	0.03–0.5		
Leuconostoc mesenteroides (1)	MDL 63,246	>128		
	MDL 62,476	>128		
	Teicoplanin	>128		
	Penicillin G	1		
	Erythromycin	1		
	Clindamycin	0.25		

[a] Two *S. acidominimus*, four *S. agalactiae*, two *S. bovis*, three *S. gordonii*, one *S. mitis*, three *S. mutans*, two *S. oralis*, three *S. salivarius*, and three *S. sanguis* isolates.
[b] Eight *Corynebacterium* sp. strain JK and four *Corynebacterium* spp.
[c] One *P. acidolactici*, two *P. pentosaceus*, and five *Pediococcus* spp.
[d] Glycopeptide-susceptible isolates: one *L. acidophilus* and two *L. delbruckii lactis* isolates.
[e] Glycopeptide-resistant isolates: one isolate each of *L. casei casei*, *L. casei rhamnosus*, *L. fermentum*, and *L. plantarum.*

TABLE 4. Activity of MDL 63,246 against 20 isolates of *N. gonorrhoeae*

Antimicrobial agent	MIC μg/ml)		
	Range	50%	90%
MDL 63,246	1–8	4	4
Penicillin G	0.016–128	2	128
Tetracycline	0.06–2	0.5	1

TABLE 5. Efficacy of MDL 63,246 in experimental septicemia in immunocompetent mice

Microorganism	Antimicrobial agent	MIC (μg/ml)	ED_{50} (mg/kg/dose) [95% confidence limits][a]
S. aureus Smith	MDL 63,246	0.06	0.035 (0.026–0.046)
	Teicoplanin	0.5	0.20 (0.16–0.24)
	Vancomycin	0.5	1.0 (1.2–0.8)
S. pyogenes L49[b]	MDL 63,246	0.016	0.03 (0.014–0.018)
	Penicillin G	0.008	0.25 (0.23–0.34)
	Teicoplanin	0.03	0.20 (0.14–0.18)
	Vancomycin	0.5	0.79 (0.9–1.1)
S. pneumoniae L44[b]	MDL 63,246	0.016	0.20 (0.18–0.22)
	Penicillin G	0.016	0.79 (0.65–0.95)
	Teicoplanin	0.06	0.31 (0.28–0.35)
	Vancomycin	0.5	0.88 (—[c])

[a] ED_{50}, 50% effective dose.
[b] In these experiments, penicillin G and vancomycin were administered twice.
[c] —, confidence limits could not be calculated.

the controls ($P < 0.01$) at all time points. Vancomycin (20 mg/kg) produced no significant drop in bacterial load. After an initial significant drop in bacterial titer for at least 6 h ($P < 0.01$ versus the control at 6 h), regrowth was observed in teicoplanin-treated (20 mg/kg) animals. On the basis of the results of the first experiment, we compared the effects of a single 20-mg/kg administration of MDL 63,246 with those of two administrations, at 2 and 6 h after infection, of vancomycin (40 mg/kg per dose) and teicoplanin (20 mg/kg per dose). Similar results were obtained with these regimens of teicoplanin and vancomycin (Fig. 3b); the regimens appeared to be more efficacious than those used in the previous experiment, because the bacterial loads in the thighs of treated animals remained significantly lower than those in the thighs of the untreated controls for up to 48 h. However, a single administration of MDL 63,246 produced a more rapid and extensive reduction in the bacterial titer (4 to 5 logs at 30 to 48 h); the bacterial loads were significantly lower ($P < 0.01$) than those in the controls and the groups treated with teicoplanin or vancomycin.

Activity of MDL 63,246 against endocarditis in rats. The results of three endocarditis experiments comparing the activities of MDL 63,246 and teicoplanin are reported in Table 7. In all of the experiments teicoplanin was administered every 12 h and MDL 63,246 was administered once daily; the first dose of both compounds was a doubled loading dose. Against *S. aureus* L1524 (for which the MICs of MDL 63,246 and teicoplanin differ by a factor of 4), the once-daily regimen of 20 mg/kg (loading dose, 40 mg/kg) of MDL 63,246 had activity in reducing the bacterial load in the heart equivalent to that of the twice-daily schedule of teicoplanin (20 mg/kg; 40-mg/kg loading dose). The groups of rats treated with either dosage of MDL 63,246 or with teicoplanin had significantly higher mean survival times and survival rates than untreated animals. Against a second isolate of *S. aureus* (isolate L561), for which the MICs of the two compounds differed by a factor of 64, a 20-mg/kg loading dose of MDL 63,246 and then 10 mg/kg daily was a more efficacious regimen than a 40 mg/kg loading dose of

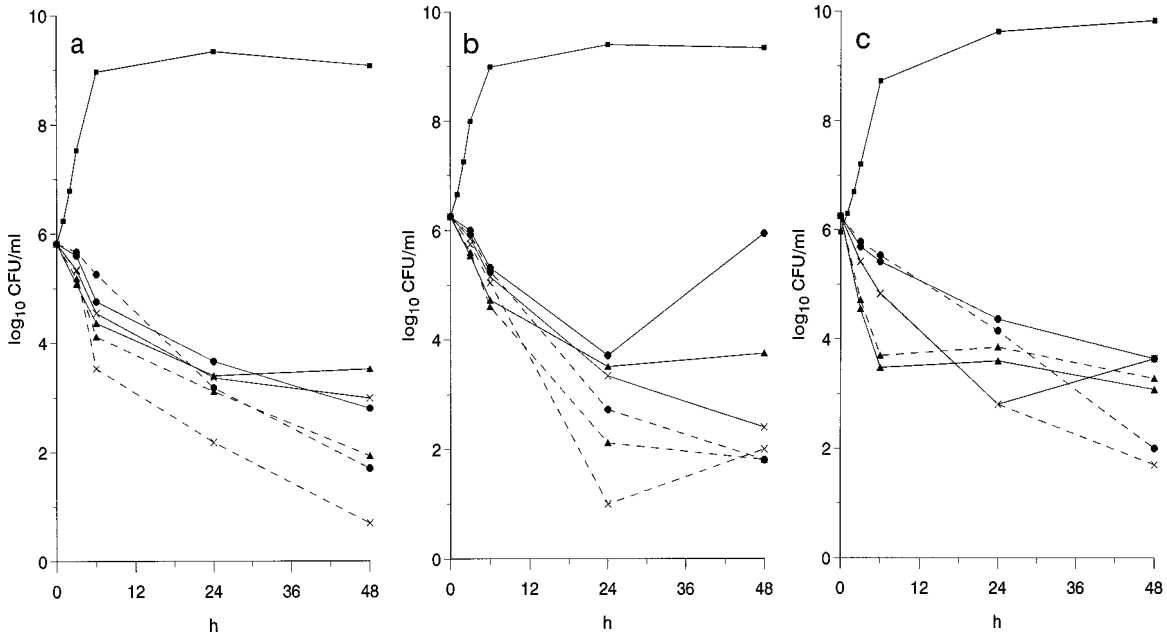

FIG. 2. Bactericidal activity of MDL 63,246 against *S. aureus* L1524 (a), L165 (b), and L1658 (c). ■, untreated control; ●, MDL 63,246 (2 and 4 μg/ml for L1524 and L1658; 1 and 2 μg/ml for L165); ×, teicoplanin (8 and 16 μg/ml); ▲, vancomycin (8 and 16 μg/ml). Solid lines, lower concentration of each compound; broken lines, higher concentration of each compound. The concentrations tested were four and eight times the MIC determined under test conditions; broth microdilution MICs were generally lower than MICs in flasks (up to eightfold).

TABLE 6. Efficacy of MDL 63,246 in experimental septicemia in neutropenic mice

Microorganism	Antimicrobial agent	MIC (μg/ml)	ED_{50} (mg/kg/dose [95% confidence limits])[a]
S. epidermidis L1480	MDL 63,246	0.13	0.58 (0.48–0.70)
	Teicoplanin	4	7.1 (5.8–8.6)
	Vancomycin	2	8.1 (7.0–9.3)
E. faecalis L1139	MDL 63,246	0.03	1.2 (0.99–1.4)
	Teicoplanin	≤0.13	0.58 (0.48–0.71)
	Vancomycin	4	9.3 (7.2–12)

[a] ED_{50}, 50% effective dose; Vancomycin was administered twice, the other antimicrobial agents were administered once.

teicoplanin and then 20 mg/kg twice daily. The efficacy of MDL 63,246 against this infection was also reflected in a significant number of animals that had no detectable bacteria in the heart at the time of death or sacrifice. In both of the experiments with *S. aureus*, the mean survival times of groups of rats treated with either dosage of MDL 63,246 or with teicoplanin were significantly longer than those of the rats in the control groups. Survival rates were significant for all treated groups in the experiment with strain L1524; in the experiment with strain L561, only the groups treated with MDL 63,246 had significant survival rates.

Against an isolate of *S. epidermidis* with intermediate susceptibility to teicoplanin, teicoplanin (loading dose of 40 mg/kg and 20 mg/kg twice daily) produced a 2-log drop in the mean heart load, although there was no statistically significant difference compared with the load in untreated controls. MDL 63,246 produced statistically significant reductions in the bacterial load at both dosages tested (loading dose of 40 mg/kg and 20 mg/kg thereafter and loading dose of 20 mg/kg and 10 mg/kg thereafter), although the titers were not significantly different from those of the group treated with teicoplanin. Only the group treated with the higher dosage of MDL 63,246 had a significant proportion of animals with no detectable bacteria in their hearts.

As expected from its very long half-life in the rat (5), the concentrations of MDL 63,246 (administered once a day) in plasma were higher than those of teicoplanin (twice daily) both at 1 h after administration and at the end of the treatment interval (Table 8).

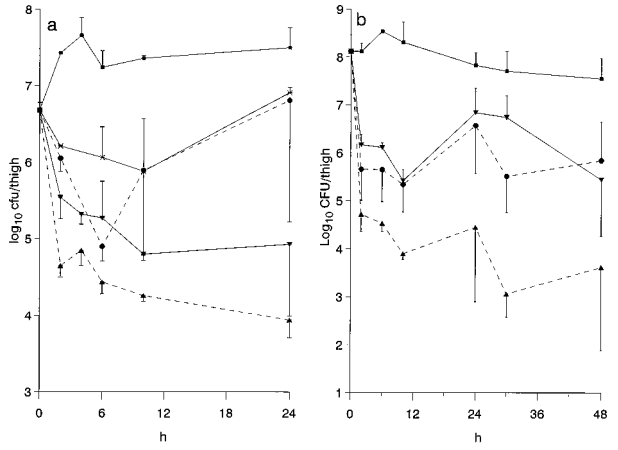

FIG. 3. Activity of MDL 63,246 against thigh muscle infection with *S. epidermidis* L1480. Datum points represent the mean number of CFU per thigh for three to five animals. The 0-h time point is just before the first treatment (2 h after infection). (a) A 24-h experiment with a single intravenous administration of antibacterial agents 2 h after infection. ■, untreated controls; ×, 20 mg of vancomycin per kg; ●, 20 mg of teicoplanin per kg; ▼, 5 mg of MDL 63,246 per kg; ▲, 20 mg of MDL 63,246 per kg. (b) A 48-h experiment. ■, untreated controls; ▼, 40 mg of vancomycin per kg at 2 and 6 h after infection; ●, 20 mg of teicoplanin per kg at 2 and 6 h after infection; ▲, 20 mg of MDL 63,246 per kg at 2 h after infection.

DISCUSSION

MDL 63,246, a semisynthetic derivative of MDL 62,476, had excellent in vitro activity against various species of glycopeptide-susceptible bacteria. In particular, it appeared to be significantly more active against staphylococci than the other natural and semisynthetic glycopeptides tested. Because the set of isolates used in the study was biased to include a high proportion of strains which were less susceptible to teicoplanin, the antistaphylococcal activity of MDL 63,246 compared with those of its parent compound MDL 62,476 and teicoplanin was particularly evident. MDL 63,246 had activity similar to those of both its parent compound and teicoplanin against vancomycin-susceptible enterococci and against VanB and VanC (teicoplanin-susceptible) isolates. Against other gram-positive genera, MDL 63,246 was as active as or somewhat more active than teicoplanin. Similar observations on the in vitro activity of MDL 63,246 have been reported by Kenny et al. (15) with U.S. clinical isolates. The bactericidal activity of MDL 63,246 against *S. aureus* compared with those of teicoplanin and vancomycin was consistent with the relative MICs of these compounds for the isolates tested.

The efficacy of MDL 63,246 in animal infection studies probably reflects both its excellent in vitro activity and its exceptionally high and prolonged levels in blood. Its 50% effective doses were lower than those of the comparison agents against staphylococcal, streptococcal, and enterococcal septicemia in immunocompetent and neutropenic mice. In the thigh infection model in neutropenic mice, a single administration of 5 mg of MDL 63,246 per kg was more efficacious than 20 mg of teicoplanin or vancomycin per kg in reducing the bacterial load of *S. epidermidis* and in preventing regrowth of this organism (which occurred by 24 h after the administration of single 20-mg/kg doses of the other agents). A single 20-mg/kg dose of MDL 63,246 rapidly reduced the bacterial load in the thighs and maintained low bacterial loads for at least 48 h. The rapidity in the reduction of the bacterial load probably reflects the in vitro activity of MDL 63,246 against the *S. epidermidis* isolate (MIC 1/16th that of vancomycin and 1/32nd that of teicoplanin), while the suppression of regrowth after administration of a single dose may also reflect the long half-life of this compound. Preliminary data indicate that levels (ca. 2 μg/ml of plasma) of MDL 63,246 greater than the MIC persist in the blood for 24 h after administration of 20 mg/kg to mice. Similar concentrations of teicoplanin were obtained in plasma at 24 h when the antibiotic was administered twice, but vancomycin was undetectable at 24 h, even after the administration of two 40-mg/kg doses.

In *S. aureus* and *S. epidermidis* endocarditis experiments in rats, MDL 63,246 treatment produced significant reductions in the bacterial load in the heart at lower dosages and with less frequent administration than teicoplanin. In the case of an *S. aureus* isolate for which the MIC of MDL 63,246 was 1/64th that of teicoplanin, daily treatment with 10 or 20 mg of MDL 63,246 per kg (loading doses 20 and 40 mg/kg, respectively) performed significantly better than teicoplanin (20 mg/kg twice a day; loading dose, 40 mg/kg) in terms of the bacterial load in the heart. In preliminary experiments with vancomycin-suscep-

TABLE 7. Efficacy of MDL 63,246 in endocarditis in rats

Strain	Agent (MIC [μg/ml])	Treatment schedule			No. of survivors/total no.	Mean ± SE survival time (days)	No. of sterile samples[a]	Mean ± SD $\log_{10}$ CFU/g of heart	
		Loading dose (mg/kg)[a]	Subsequent doses (mg/kg)	Dose interval (h)				All animals	Animals that were early deaths
S. aureus L1524	None				0/9	3.0 ± 0.1	0	9.3 ± 0.4	9.3 ± 0.4
	MDL 63,246 (0.13)	20	10	24	6/8[b]	4.9 ± 0.1[c]	1	5.6 ± 1.7[b]	5.8 ± 0.9
	MDL 63,246	40	20	24	5/9[d]	4.4 ± 0.2[c]	4	4.1 ± 2.6[c]	5.2 ± 2.5
	Teicoplanin (0.5)	40	20	12	9/9[c]	>5.7[c]	2	4.5 ± 2.2[c]	
	Pretreatment[e]							7.3 ± 0.5	
S. aureus L561	None				0/9	2.9 ± 0.1	0	8.3 ± 0.4	8.3 ± 0.4
	MDL 63,246 (0.13)	20	10	24	5/8[d]	5.2 ± 0.4[c]	6[d]	2.9 ± 1.3[f]	3.2 ± 1.8
	MDL 63,246	40	20	24	7/10[b]	5.3 ± 0.3[c]	6[d]	3.0 ± 1.2[f]	4.5 ± 1.2
	Teicoplanin (8)	40	20	12	4/9	4.4 ± 0.3[b]	0	6.4 ± 1.1[b]	5.8 ± 1.2
	Pretreatment[e]							6.4 ± 0.3	
S. epidermidis L537	None				3/10	4.6 ± 0.4	0	6.8 ± 0.4	6.9 ± 0.5
	MDL 63,246 (0.13)	20	10	24	4/10	4.0 ± 0.4	3	4.4 ± 1.9[b]	4.8 ± 2.0
	MDL 63,246	40	20	24	7/11	4.5 ± 0.3	5[c]	3.6 ± 1.8[c]	4.1 ± 2.2
	Teicoplanin (16)	40	20	12	4/12	4.7 ± 0.4	1	4.9 ± 1.7	5.1 ± 1.7
	Pretreatment[e]							6.7 ± 0.2	

[a] Detection limit of 2.3 $\log_{10}$ CFU/g of heart.
[b] $P < 0.01$.
[c] $P < 0.001$ versus untreated controls.
[d] $P < 0.05$.
[e] Determined in a separate group of five infected animals just before the first scheduled treatment.
[f] $P < 0.001$ versus untreated controls and versus teicoplanin group.

tible enterococci (data not shown), MDL 63,246 appeared to be efficacious at lower dosages and at longer treatment intervals than either teicoplanin or vancomycin. Although MDL 63,246 showed moderate in vitro activity against VanA (teicoplanin-resistant) isolates (as was also observed by Kenny et al. [15]), the results of a preliminary endocarditis experiment with a VanA isolate suggest that MDL 63,246 might not be sufficiently active against these organisms in vivo; however, the potential of combination therapy with other classes of antibiotics has not yet been explored.

The results of these studies suggest that MDL 63,246 or other semisynthetic glycopeptides with similar antibacterial activities and pharmacokinetics could be therapeutically efficacious at lower dosages and longer treatment intervals than those currently used for vancomycin and teicoplanin.

TABLE 8. Concentrations of antimicrobial agents in plasma in the endocarditis experiments

Antimicrobial agent	Dose (mg/kg)[a]	Treatment interval (h)	Mean ± SD concn in plasma (μg/ml [no. of rats])[b]	
			1 h[c]	Trough[d]
MDL 63,246	10	24	95 ± 22 (15)	32 ± 19 (15)
MDL 63,246	20	24	170 ± 75 (19)	62 ± 19 (19)
Teicoplanin	20	12	43 ± 7 (8)	4 ± 1 (17)[e]

[a] The first dose was a doubled loading dose.
[b] The data are for animals that survived to the end of the experiments.
[c] On day 2 of treatment: 1 h after administration of the second dose of MDL 63,246 and 1 h after administration of the third dose of teicoplanin. The 1-h concentrations in the plasma of animals that died before the end of the experiment did not differ significantly from those reported.
[d] Trough levels were determined upon sacrificing these animals after allowing the normal between-dose interval after the last treatment: 24 h after administration of the fifth dose of MDL 63,246 and 12 h after administration of the 10th dose of teicoplanin.
[e] Data are from a single experiment.

REFERENCES

1. **Aubert, G., S. Passot, F. Lucht, and G. Dorche.** 1990. Selection of vancomycin- and teicoplanin-resistant *Staphylococcus haemolyticus* during teicoplanin treatment of *S. epidermidis* infection. J. Antimicrob. Chemother. **25:**491–493.
2. **Bartoloni, A., M. G. Colao, A. Orsi, R. Deo, E. Giganti, and F. Parenti.** 1990. In-vitro activity of vancomycin, teicoplanin, daptomycin, ramoplanin, MDL 62873 and other agents against staphylococci, enterococci and *Clostridium difficile*. J. Antimicrob. Chemother. **26:**627–633.
3. **Berti, M., G. P. Candiani, M. Borgonovi, P. Landini, F. Ripamonti, R. Scotti, L. Cavenaghi, M. Denaro, and B. P. Goldstein.** 1992. Antimicrobial activity of MDL 62,873, a semisynthetic derivative of teicoplanin, in vitro and in experimental infections. Antimicrob. Agents Chemother. **36:**446–452.
4. **Biavasco, F., R. Lupidi, and P. E. Varaldo.** 1992. In vitro activities of three semisynthetic amide derivatives of teicoplanin, MDL 62208, MDL 62211, and MDL 62873. Antimicrob. Agents Chemother. **36:**331–338.
5. **Borgonovi, M., P. Kaltofen, and L. A. Cavenaghi.** 1993. Pharmacokinetics of MDL 63,246, a new semisynthetic glycopeptide antibiotic, in rat and rabbit, abstr. 447, p. 200. *In* Program and abstracts of the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy. American Society for Microbiology, Washington, D.C.
6. **Brumfitt, W., P. A. C. Maple, and J. M. T. Hamilton-Miller.** 1990. Ramoplanin versus methicillin-resistant *Staphylococcus aureus*: *in vitro* experience. Drugs Exp. Clin. Res. **16:**377–383.
7. **Campoli-Richards, D. M., R. N. Brogden, and D. Faulds.** 1990. Teicoplanin. A review of its antibacterial activity, pharmacokinetic properties and therapeutic potential. Drugs **40:**449–486.
8. **Clark, N. C., R. C. Cooksey, B. C. Hill, J. M. Swenson, and F. C. Tenover.** 1993. Characterization of glycopeptide-resistant enterococci from U.S. hospitals. Antimicrob. Agents Chemother. **37:**2311–2317.
9. **Finney, D. J.** 1952. The Spearman-Kärber method, p. 524–530. *In* D. J. Finney (ed.), Statistical method in biological assay. Charles Griffin & Company Limited, London.
10. **Goldstein, B. P., R. Rosina, and F. Parenti.** 1994. Teicoplanin, p. 273–307. *In* R. Nagarajan (ed.), Glycopeptide antibiotics. Marcel Dekker, Inc., New York.
11. **Goldstein, B. P., E. Selva, L. Gastaldo, M. Berti, R. Pallanza, F. Ripamonti, P. Ferrari, M. Denaro, V. Arioli, and G. Cassani.** 1987. A40926, a new glycopeptide antibiotic with anti-*Neisseria* activity. Antimicrob. Agents Chemother. **31:**1961–1966.
12. **Goldstein, F. W., A. Coutrot, A. Sieffer, and J. F. Acar.** 1990. Percentages and distributions of teicoplanin- and vancomycin-resistant strains among coagulase-negative staphylococci, Antimicrob. Agents Chemother. **34:**899–900.
13. **Gordon, S., J. M. Swenson, B. C. Hill, N. E. Pigott, R. R. Facklam, R. C. Cooksey, C. Thornsberry, Enterococcal Study Group, W. R. Jarvis, and F. C.**

Tenover. 1992. Antimicrobial susceptibility patterns of common and unusual species of enterococci causing infections in the United States. J. Clin. Microbiol. **30:**2373–2378.
14. **Jones, R. N., F. W. Goldstein, and X. Y. Zhou.** 1991. Activities of two new teicoplanin amide derivatives (MDL 62211 and MDL 62873) compared with activities of teicoplanin and vancomycin against 800 recent staphylococcal isolates from France and the United States. Antimicrob. Agents Chemother. **35:**584–586.
15. **Kenny, M. T., M. A. Brackman, and J. K. Dulworth.** 1995. In vitro activity of the semisynthetic glycopeptide amide MDL 63,246. Antimicrob. Agents Chemother. **39:**1589–1590.
16. **Leclercq, R., E. Derlot, J. Duval, and P. Courvalin.** 1988. Plasmid-mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. N. Engl. J. Med. **319:**157–161.
17. **Murray, B. E.** 1990. The life and times of the enterococcus. Clin. Microbiol. Rev. **3:**46–65.
18. **National Committee for Clinical Laboratory Standards.** 1990. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 2nd ed. Approved standard M7-A2. National Committee for Clinical Laboratory Standards, Villanova, Pa.
19. **SAS Institute, Inc.** 1990. SAS user's guide: statistics, version 6.06. SAS Institute, Inc., Cary, N.C.
20. **Schwalbe, R. S., J. T. Stapleton, and P. H. Gilligan.** 1987. Emergence of vancomycin resistance in coagulase-negative staphylococci, N. Engl. J. Med. **316:**927–931.
21. **Scotti, R., J. K. Dulworth, M. T. Kenny, and B. P. Goldstein.** 1993. Effect of protein on ramoplanin broth microdilution MICs. Diagn. Microbiol. Infect. Dis. **17:**209–211.
22. **Selva, E., B. P. Goldstein, P. Ferrari, R. Pallanza, E. Riva, M. Berti, A. Borghi, G. Beretta, R. Scotti, G. Romanò, G. Cassani, V. Arioli, and M. Denaro.** 1988. A40926 aglycone and pseudoaglycones: preparation and biological activity. J. Antibiot. **41:**1243–1252.
23. **Uttley, A. H. C., R. C. George, J. Naidoo, N. Woodford, A. P. Johnson, C. H. Collins, D. Morrison, A. J. Gilfillan, L. E. Fitch, and J. Heptonstall.** 1989. High-level vancomycin resistant enterococci causing hospital infections. Epidemiol. Infect. **103:**173–181.
24. **Veach, L. A., M. A. Pfaller, M. Barrett, F. P. Koontz, and R. P. Wenzel.** 1990. Vancomycin resistance in *Staphylococcus haemolyticus* causing colonization and bloodstream infection. J. Clin. Microbiol. **28:**2064–2068.
25. **Woodford, N., A. P. Johnson, D. Morrison, A. T. L. Chin, J. R. Stephenson, and R. C. George.** 1990. Two distinct forms of vancomycin resistance amongst enterococci in the UK. Lancet **335:**226.