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In Vitro Activities of Two Glycylcyclines against Gram-Positive Bacteria

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The glycylcyclines designated CL 329,998 and CL 331,002 are *N,N*-dimethylglycylamido derivatives of minocycline and 6-demethyl-6-deoxytetracycline, respectively. In vitro activities of these two antimicrobial agents were compared with those of tetracycline, minocycline, and seven other antimicrobial agents against 412 gram-positive organisms. Both new drugs were significantly more active than minocycline against methicillin-resistant *Staphylococcus aureus* (MICs for 90% of isolates tested, 0.25 and 0.5 μg/ml versus 4 μg/ml). CL 329,998 inhibited all streptococci, lactobacilli, and *Leuconostoc* spp. at concentrations of ≤0.5 μg/ml, with CL 331,002 slightly less active against some species. All enterococci, including minocycline-resistant and multidrug-resistant isolates, were inhibited at ≤0.5- and ≤1.0-μg/ml concentrations of the new drugs, respectively. Only bacteriostatic activity was evident by time-kill curves. The two glycylcyclines demonstrated activities in vitro that were superior to those of minocycline against several gram-positive bacterial species, and at relatively low concentrations, they inhibited isolates resistant to both tetracycline and minocycline.

Although the tetracyclines remain valuable agents for the treatment of a variety of infectious diseases, resistance to this class of antibiotics limits their use against a number of important gram-positive bacterial pathogens. The reported rates of resistance to tetracycline among coagulase-positive and -negative staphylococci, pneumococci, and group A streptococci have varied widely on the basis of geographic locale and year of isolation (7). Of recent isolates encountered at our hospital, only 33% of enterococci were fully susceptible to tetracycline. While the spread of tetracycline resistance determinants among medically important bacteria threatens to limit further the utility of these drugs, it has been proposed that novel analogs might be developed with activity against organisms resistant to older compounds of this class (1, 15). The compounds CL 329,998 and CL 331,002 are *N,N*-dimethylglycylamido derivatives of minocycline and 6-demethyl-6-deoxytetracycline, respectively, and are referred to as glycylcyclines (5, 16). The present study examined the in vitro activities of these glycylcyclines against gram-positive bacterial isolates, including strains resistant to β-lactams, glycopeptides, tetracycline, and minocycline.

(This work was presented in part at the 33rd Interscience Conference on Antimicrobial Agents and Chemotherapy [5].)

MATERIALS AND METHODS

Bacterial strains. Routine clinical isolates of bacteria collected at the Massachusetts General Hospital or New England Deaconess Hospital were included without regard to tetracycline resistance patterns. Strains reflecting unusual resistance traits, including glycopeptide-resistant or β-lactamase-producing enterococci, *Enterococcus avium*, and *Enterococcus raffinosus*, and β-lactam-resistant streptococci had been referred to our laboratory from various sources (2, 4, 13).

Antimicrobial agents. CL 329,998, CL 331,002, and minocycline were provided by Lederle Laboratories, American Cyanamid Co., Pearl River, N.Y. Tetracycline, ampicillin, and oxacillin were purchased from Sigma Chemical Company, St. Louis, Mo. Other antimicrobial agents provided as powders were generous gifts of their manufacturers: clindamycin (The Upjohn Co., Kalamazoo, Mich.), ciprofloxacin (Miles, Inc., West Haven, Conn.), imipenem (Merck Sharp & Dohme, West Point, Pa.), erythromycin (Abbott Laboratories, North Chicago, Ill.), and vancomycin (Eli Lilly & Co., Indianapolis, Ind.).

Susceptibility and time-kill studies. The activities of the antimicrobial agents were determined by an agar dilution method (12), utilizing Mueller-Hinton II agar (Becton Dickinson, Cockeysville, Md.), which was supplemented with 5% sheep blood for streptococci and corynebacteria. Inocula of approximately 10^4 CFU per spot were applied to the surfaces of plates, which were incubated for 24 h at 35°C in ambient air or in 5% CO_2 (for *Lactobacillus*, *Leuconostoc*, and *Pediococcus* spp.). As controls, *Staphylococcus aureus* ATCC 29213 and *Escherichia coli* ATCC 25922 were included in all plates. Time-kill studies were performed in 20-ml volumes of glucose-phosphate broth (Adams Scientific, Inc., West Warwick, R.I.) with 0.1% citrate and with or without antimicrobial agents. Flasks were inoculated with stationary-phase cultures of bacteria, yielding final cell densities of approximately 10^6 CFU/ml, and sampled at 0, 4, and 24 h of incubation in room air. Samples were serially diluted, and colony counts were performed in duplicate.

RESULTS

Susceptibility studies. The results of susceptibility studies are shown in Table 1. The glycylcyclines were fourfold less active than minocycline against oxacillin-susceptible *S. aureus* by comparing MICs for 90% of isolates tested and only twofold less active by comparing geometric means of MICs (0.25 versus 0.125 μg/ml), but the glycylcyclines were considerably more active than minocycline against oxacillin-resistant strains. Six strains of borderline oxacillin-susceptible *S. aureus* not included in Table 1 were inhibited by CL 329,998 at 0.25 μg/ml, CL 331,002 at 0.12 to 0.25 μg/ml, and minocycline at 0.12

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TABLE 1. Comparative in vitro activities of CL 329,998 and CL 331,002

Organism (no.)	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
Staphylococcus aureus				
Methicillin-susceptible (30)	CL 329,998	0.12–4	0.25	0.5
	CL 331,002	0.12–8	0.25	0.5
	Minocycline	0.06–1	0.12	0.12
	Tetracycline	0.12–>128	0.5	1
	Ciprofloxacin	0.25–>128	0.5	2
	Imipenem	0.008–0.03	0.03	0.03
	Ampicillin	0.12–64	2	8
	Oxacillin	0.12–0.5	0.5	0.5
	Clindamycin	0.06–>128	0.12	0.25
	Erythromycin	0.12–>128	0.25	16
	Vancomycin	0.5–2	1	1
Methicillin-resistant (32)	CL 329,998	0.12–1	0.25	0.25
	CL 331,002	0.25–2	0.25	0.5
	Minocycline	0.12–8	0.12	4
	Tetracycline	0.5–64	1	64
	Ciprofloxacin	0.25–32	1	32
	Imipenem	0.25–>128	64	128
	Oxacillin	16–>128	>128	>128
	Clindamycin	0.12–>128	>128	>128
	Erythromycin	0.5–>128	>128	>128
	Vancomycin	0.5–2	1	2
Coagulase-negative staphylococci				
Methicillin-susceptible (23)	CL 329,998	0.12–4	0.25	2
	CL 331,002	0.12–8	0.25	8
	Minocycline	0.06–8	0.06	0.25
	Tetracycline	0.12–>128	0.25	>128
	Ciprofloxacin	0.12–128	0.5	64
	Imipenem	≤0.004–1	0.03	0.25
	Oxacillin	0.016–2	0.25	2
	Clindamycin	0.06–>128	0.12	0.5
	Erythromycin	0.12–>128	16	>128
	Vancomycin	0.5–2	1	2
Methicillin-resistant (37)	CL 329,998	0.12–16	1	4
	CL 331,002	0.12–32	1	16
	Minocycline	0.06–2	0.5	1
	Tetracycline	0.12–>128	4	>128
	Ciprofloxacin	0.12–128	0.5	64
	Imipenem	0.5–>128	16	128
	Oxacillin	4–>128	32	>128
	Clindamycin	0.12–>128	>128	>128
	Erythromycin	0.25–>128	>128	>128
	Vancomycin	0.5–4	2	4
Streptococcus pneumoniae				
Penicillin-susceptible (10)	CL 329,998	0.06–0.12	0.06	0.12
	CL 331,002	0.06–0.12	0.12	0.12
	Minocycline	0.12–0.25	0.25	0.25
	Tetracycline	0.25–0.5	0.5	0.5
	Ciprofloxacin	1–4	2	4
	Imipenem	≤0.004–0.008	0.008	0.008
	Ampicillin	0.03	0.03	0.03
	Oxacillin	0.06–0.12	0.12	0.12
	Clindamycin	0.06–0.12	0.06	0.12
	Erythromycin	0.016–0.06	0.03	0.06
	Vancomycin	0.12–0.5	0.25	0.5
Penicillin-resistant (11)	CL 329,998	0.016–0.5	0.12	0.5
	CL 331,002	0.06–1	0.12	0.5
	Minocycline	0.12–64	2	32
	Tetracycline	0.12–>128	8	>128
	Ciprofloxacin	1–8	2	4
	Imipenem	0.008–0.5	0.25	0.5

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TABLE 1—*Continued*

Organism (no.)	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
	Ampicillin	0.03–8	2	8
	Oxacillin	1–32	16	32
	Clindamycin	0.008–128	0.12	32
	Erythromycin	0.016–>128	16	>128
	Vancomycin	0.12–2	0.5	1
Viridans group streptococci				
Penicillin-susceptible (22)	CL 329,998	0.06–1	0.12	0.25
	CL 331,002	0.12–4	0.25	1
	Minocycline	0.12–16	0.5	8
	Tetracycline	1–64	2	32
	Ciprofloxacin	0.5–2	1	2
	Imipenem	≤0.004–0.12	0.03	0.06
	Ampicillin	0.06–4	0.25	0.5
	Oxacillin	0.12–1	0.5	1
	Clindamycin	≤0.004–0.12	0.03	0.12
	Erythromycin	0.016–0.06	0.03	0.06
	Vancomycin	0.5–1	0.5	1
Penicillin-resistant (10)	CL 329,998	0.12–0.5	0.25	0.25
	CL 331,002	0.12–1	0.5	1
	Minocycline	4–64	32	64
	Tetracycline	8–>128	128	>128
	Ciprofloxacin	1–8	4	8
	Imipenem	0.12–4	1	1
	Ampicillin	2–16	16	16
	Oxacillin	8–64	32	64
	Clindamycin	0.016–128	0.06	64
	Erythromycin	0.03–>128	2	>128
	Vancomycin	0.5–1	0.5	1
Group A streptococci (10)	CL 329,998	0.12–0.25	0.25	0.25
	CL 331,002	0.12–0.5	0.5	0.5
	Minocycline	0.25–0.5	0.5	0.5
	Tetracycline	0.25–1	1	1
	Ciprofloxacin	0.5–2	0.5	2
	Imipenem	≤0.004–0.008	≤0.004	0.008
	Ampicillin	0.016–0.06	0.03	0.03
	Oxacillin	0.03–0.06	0.06	0.06
	Clindamycin	0.03–0.12	0.03	0.12
	Erythromycin	0.06	0.06	0.06
	Vancomycin	0.25–1	0.5	1
Group B streptococci (10)	CL 329,998	0.12–0.25	0.25	0.25
	CL 331,002	0.25–1	0.25	1
	Minocycline	0.5–64	32	64
	Tetracycline	1–128	64	128
	Ciprofloxacin	2	2	2
	Imipenem	0.008–0.03	0.016	0.03
	Ampicillin	0.12–0.25	0.12	0.25
	Oxacillin	0.25–2	0.5	0.5
	Clindamycin	0.06–0.12	0.12	0.12
	Erythromycin	0.06	0.06	0.06
	Vancomycin	0.5–1	1	1
Group C and G streptococci (10)	CL 329,998	0.25–0.5	0.25	0.5
	CL 331,002	0.12–1	0.25	1
	Minocycline	0.12–32	0.5	16
	Tetracycline	0.5–>128	2	128
	Ciprofloxacin	0.5–2	1	2
	Imipenem	≤0.004–0.016	0.008	0.016
	Ampicillin	0.016–0.12	0.016	0.06
	Oxacillin	0.03–0.25	0.06	0.06
	Clindamycin	0.06–0.25	0.12	0.12
	Erythromycin	0.06–0.12	0.06	0.06
	Vancomycin	0.25–1	0.25	1

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TABLE 1—*Continued*

Organism (no.)	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
Enterococcus faecalis (16)	CL 329,998	0.12–0.25	0.25	0.25
	CL 331,002	0.12–0.5	0.5	0.5
	Minocycline	0.25–32	16	32
	Tetracycline	1–128	64	128
	Ciprofloxacin	1–8	2	4
	Imipenem	0.5–2	2	2
	Ampicillin	0.5–2	1	2
	Clindamycin	16–>128	32	>128
	Erythromycin	0.25–>128	4	>128
	Vancomycin	1–2	1	2
Vancomycin-resistant (14)	CL 329,998	0.12–0.5	0.25	0.5
	CL 331,002	0.25–1	0.5	1
	Minocycline	0.12–32	16	16
	Tetracycline	0.5–>128	>128	>128
	Ciprofloxacin	1–128	2	128
	Imipenem	1–8	2	4
	Ampicillin	0.5–2	2	2
	Clindamycin	16–>128	>128	>128
	Erythromycin	4–>128	>128	>128
	Vancomycin	16–>128	>128	>128
Highly gentamicin resistant (14)	CL 329,998	0.12–0.25	0.25	0.25
	CL 331,002	0.25–1	0.5	0.5
	Minocycline	0.12–32	0.25	32
	Tetracycline	1–128	1	64
	Ciprofloxacin	0.5–128	2	64
	Imipenem	1–4	2	4
	Ampicillin	1–4	2	2
	Clindamycin	32–>128	>128	>128
	Erythromycin	4–>128	>128	>128
	Vancomycin	1–4	1	2
Highly gentamicin-resistant, β-lactamase producing (15)	CL 329,998	0.12–0.25	0.12	0.25
	CL 331,002	0.12–0.25	0.12	0.25
	Minocycline	4–16	8	16
	Tetracycline	32–128	128	128
	Ciprofloxacin	1	1	1
	Imipenem	1–2	1	2
	Ampicillin	1	1	1
	Clindamycin	8–>128	>128	>128
	Erythromycin	2–>128	32	>128
	Vancomycin	0.5–2	1	1
Enterococcus faecium Ampicillin-resistant (11)	CL 329,998	0.06–0.12	0.12	0.12
	CL 331,002	0.12–0.25	0.12	0.25
	Minocycline	0.12–32	8	32
	Tetracycline	0.25–128	32	128
	Ciprofloxacin	2–8	8	8
	Imipenem	128–>128	128	>128
	Ampicillin	32–>128	64	128
	Clindamycin	0.12–>128	>128	>128
	Erythromycin	2–>128	>128	>128
	Vancomycin	0.5–4	0.5	1
Highly gentamicin-resistant (10)	CL 329,998	0.06–0.12	0.06	0.12
	CL 331,002	0.06–0.25	0.06	0.25
	Minocycline	0.03–32	0.06	32
	Tetracycline	0.25–128	0.5	128
	Ciprofloxacin	1–32	2	8
	Imipenem	128–>128	>128	>128
	Ampicillin	64–>128	64	>128
	Clindamycin	0.12–>128	>128	>128
	Erythromycin	0.5–>128	>128	>128
	Vancomycin	0.5–1	0.5	1

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TABLE 1—*Continued*

Organism (no.)	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
Vancomycin-resistant (18)	CL 329,998	0.03–0.25	0.12	0.25
	CL 331,002	0.06–0.5	0.12	0.25
	Minocycline	0.03–32	0.12	32
	Tetracycline	0.25–>128	0.5	128
	Ciprofloxacin	2–16	4	8
	Imipenem	0.12–>128	>128	>128
	Ampicillin	0.25–>128	64	>128
	Clindamycin	0.06–>128	>128	>128
	Erythromycin	2–>128	>128	>128
	Vancomycin	8–>128	>128	>128
Enterococcus avium (10)	CL 329,998	0.06	0.06	0.06
	CL 331,002	0.06–0.12	0.12	0.12
	Minocycline	0.12–32	8	16
	Tetracycline	0.5–>128	64	>128
	Ciprofloxacin	1–2	1	2
	Imipenem	0.5–1	0.5	1
	Ampicillin	0.5–1	0.5	1
	Clindamycin	2–>128	4	>128
	Erythromycin	0.25–>128	0.5	>128
	Vancomycin	0.25–0.5	0.5	0.5
Enterococcus raffinosus (10)	CL 329,998	0.06	0.06	0.06
	CL 331,002	0.12–0.25	0.12	0.12
	Minocycline	0.12–32	2	32
	Tetracycline	0.5–128	16	128
	Ciprofloxacin	0.5–2	1	1
	Imipenem	32–>128	64	>128
	Ampicillin	16–64	16	32
	Clindamycin	4–>128	128	>128
	Erythromycin	0.12–>128	>128	>128
	Vancomycin	0.5–1	1	1
Enterococcus casseliflavus (5)	CL 329,998	0.06–0.12		
	CL 331,002	0.25		
	Minocycline	0.03–16		
	Tetracycline	2–64		
	Ciprofloxacin	0.5–2		
	Imipenem	0.25–1		
	Ampicillin	0.25–2		
	Oxacillin	8–16		
	Clindamycin	8–>128		
	Erythromycin	1–>128		
	Vancomycin	1–4		
Enterococcus gallinarum (5)	CL 329,998	0.06–0.25		
	CL 331,002	0.25–1		
	Minocycline	0.06–32		
	Tetracycline	1–128		
	Ciprofloxacin	0.5–4		
	Imipenem	0.5–2		
	Ampicillin	0.5–2		
	Oxacillin	16–64		
	Clindamycin	16		
	Erythromycin	0.12–2		
	Vancomycin	4–8		
Listeria monocytogenes (20)	CL 329,998	0.12–0.25	0.12	0.25
	CL 331,002	0.12–0.5	0.25	0.5
	Minocycline	0.06–0.12	0.12	0.12
	Tetracycline	0.5–4	2	2
	Ciprofloxacin	0.5–4	1	2
	Imipenem	0.03–0.5	0.12	0.25
	Ampicillin	0.06–1	1	1
	Oxacillin	0.5–8	8	8
	Clindamycin	2–8	4	8

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TABLE 1—*Continued*

Organism (no.)	Antimicrobial agent	MIC (μg/ml)[a]		
		Range	50%	90%
	Erythromycin	0.12–0.5	0.5	0.5
	Vancomycin	0.25–1	1	1
JK diphtheroids (23)	CL 329,998	0.25–4	0.5	2
	CL 331,002	0.12–8	0.5	4
	Minocycline	0.25–16	0.5	4
	Tetracycline	0.5–>128	2	64
	Ciprofloxacin	0.25–128	0.5	32
	Imipenem	0.06–>128	128	>128
	Ampicillin	1–>128	>128	>128
	Oxacillin	8–>128	>128	>128
	Clindamycin	0.25–>128	128	>128
	Erythromycin	0.06–32	1	8
	Vancomycin	0.5–1	0.5	1
Lactobacillus spp. (10)	CL 329,998	0.016–0.25	0.12	0.25
	CL 331,002	0.03–0.5	0.25	0.25
	Minocycline	0.016–8	0.5	1
	Tetracycline	0.12–16	4	16
	Ciprofloxacin	1–8	2	8
	Imipenem	0.03–8	1	2
	Ampicillin	0.12–8	2	2
	Oxacillin	2–64	16	16
	Clindamycin	0.03–0.5	0.12	0.5
	Erythromycin	0.06–0.5	0.25	0.5
	Vancomycin	>128	>128	>128
Leuconostoc spp. (10)	CL 329,998	0.06–0.25	0.12	0.25
	CL 331,002	0.12–0.5	0.25	0.5
	Minocycline	0.25–1	0.5	1
	Tetracycline	1–16	4	8
	Ciprofloxacin	1–16	2	8
	Imipenem	0.06–8	2	2
	Ampicillin	0.5–2	0.5	1
	Oxacillin	4–16	4	4
	Clindamycin	0.016–0.5	0.06	0.25
	Erythromycin	0.12–0.5	0.12	0.25
	Vancomycin	>128	>128	>128
Pediococcus spp. (8)	CL 329,998	0.25–1	0.5	1
	CL 331,002	1–2	2	2
	Minocycline	1–8	4	8
	Tetracycline	8–64	64	64
	Ciprofloxacin	32–64	32	64
	Imipenem	0.25–1	0.25	1
	Ampicillin	2–4	2	4
	Oxacillin	8–16	8	16
	Clindamycin	0.03–0.06	0.06	0.06
	Erythromycin	0.25–0.5	0.25	0.5
	Vancomycin	>128	>128	>128

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

μg/ml. The new compounds were less active than minocycline but more active than tetracycline against coagulase-negative staphylococci. The minocycline derivative was fourfold more active than CL 331,002 against both oxacillin-susceptible and -resistant coagulase-negative staphylococci. For the two new agents, the geometric means of MICs against tetracycline-resistant strains of staphylococci were five- to eightfold higher than for tetracycline-susceptible strains (Fig. 1). No isolates of staphylococci in this collection were resistant to minocycline.

All strains of enterococci, including minocycline-resistant and vancomycin-resistant isolates, were inhibited by CL 329,998 and CL 331,002 at ≤0.5 and ≤1.0 μg/ml, respectively. The glycylcyclines were the only drugs tested which inhibited some isolates of vancomycin-resistant *Enterococcus faecium* at concentrations likely to be achievable in serum. The mean MICs of the new compounds against isolates resistant to tetracycline were virtually identical to those of susceptible organisms, while the mean MICs against enterococci resistant to both tetracycline and minocycline remained within 1 twofold dilution of those of organisms resistant to neither older agent.

CL 329,998 inhibited all streptococcal isolates at ≤0.5 μg/ml. CL 331,002 was only slightly less active on the basis of the concentrations inhibiting 90% of isolates (0.12 to 1.0 μg/ml), and it inhibited all streptococci, including minocycline-

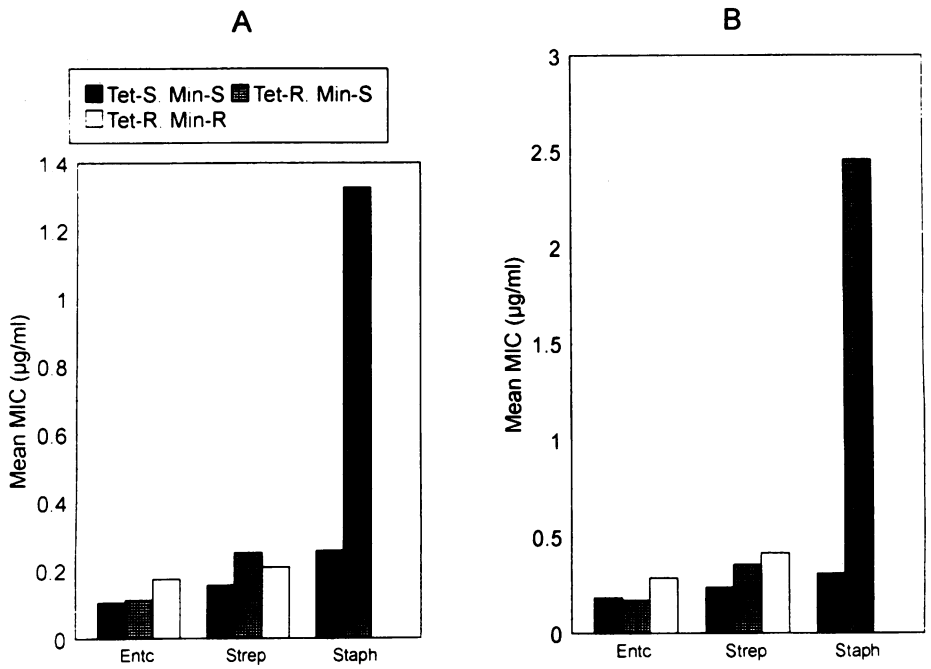

FIG. 1. MICs (geometric means) of CL 329,998 (A) and CL 331,002 (B) against gram-positive cocci grouped on the basis of resistance (R) (MIC >8 μg/ml) or susceptibility (S) to minocycline (Min) or tetracycline (Tet). Entc, enterococci; Strep, streptococci; Staph, staphylococci.

and tetracycline-resistant strains at ≤4 μg/ml. For six of the seven groups of strains shown in Table 1, the maximal MICs were ≤1.0 μg/ml. Resistance to tetracycline or to both tetracycline and minocycline had little impact on the mean MICs of the new agents. In contrast, the mean MIC of minocycline against tetracycline-resistant (but not minocycline-resistant) streptococci was 2.8 μg/ml compared with 0.4 μg/ml for tetracycline-susceptible strains.

Two strains of *Erysipelothrix* spp. were inhibited at 0.12 to 0.25 μg/ml of the new agents (minocycline MICs, 0.008 to 0.12 μg/ml). The new minocycline derivative was four- to eightfold more active than minocycline against the intrinsically vancomycin-resistant *Lactobacillus*, *Pediococcus*, and *Leuconostoc* species, some of which were tetracycline resistant. Minocycline was fourfold more active than CL 331,002 against *Listeria monocytogenes*, but even the latter was fourfold more active than tetracycline. Only vancomycin demonstrated greater activity against the corynebacteria than did the novel compounds.

Time-kill studies. Time-kill studies were performed against five vancomycin-resistant enterococci (three *Enterococcus faecalis* and two *E. faecium*) and one methicillin-resistant isolate of *S. aureus* exposed to the two new glycylcyclines at 2 μg/ml, which represents 4- to 16-fold the MICs of these agents against the strains tested. An approximately 1.5 $\log_{10}$ CFU/ml reduction in viable cells at 24 h was seen with each drug against the methicillin-resistant *S. aureus* strain. No significant killing effect was seen against the enterococci either, with final colony counts varying from the starting bacterial density by <0.5 to 1.25 $\log_{10}$ CFU/ml (Fig. 2). Minocycline at 2 μg/ml was also tested against the four strains of enterococci and one methicillin-resistant *S. aureus* susceptible to this agent, and in no case was this observed to have greater killing activity than the new drugs.

DISCUSSION

Resistance to currently available antimicrobial agents, which is encountered with increasing frequency among some groups of gram-positive bacteria, already poses significant clinical difficulties. Outbreaks of infection or colonization with methicillin-resistant *S. aureus* have occurred in a number of acute and long-term health care settings (10). Because many strains of methicillin-resistant *S. aureus* are also resistant to a number of other antibiotics as well (8, 17), vancomycin is generally regarded as the drug of choice for treatment of serious

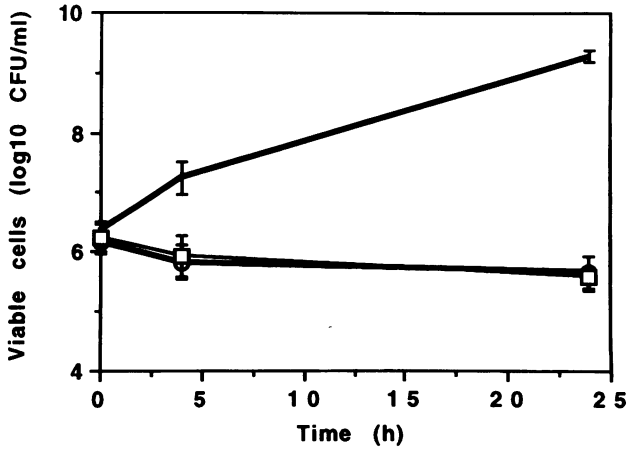

FIG. 2. Bacteriostatic effect of CL 329,998 (○) and CL 331,002 (□), each at 2 μg/ml, against five enterococcal isolates. Datum points and error bars represent the means ± standard errors of the means of viable cells ($\log_{10}$ CFU/ml) at 4- and 24-h sampling times. Growth in the absence of antimicrobial agents is also shown (——).

infections caused by these organisms. However, this drug is not tolerated by some patients. Minocycline, to which most isolates of *S. aureus* are susceptible (17), has been used effectively in treatment of infections caused by methicillin-resistant strains (18). The substantially higher potencies of CL 329,998 and CL 331,002 compared with minocycline against these organisms suggest a potentially important role for these new drugs in therapy of infections caused by methicillin-resistant *S. aureus*. Any advantage of the glycylcyclines relative to minocycline was lost against methicillin-susceptible strains of *S. aureus*, and curiously, the new drugs were substantially less potent than minocycline against coagulase-negative staphylococci.

Rates of resistance to tetracyclines among clinical isolates of *Streptococcus pneumoniae* have varied widely (7). At two Paris hospitals, resistance frequencies more than tripled from 14% in 1970 to 46.5% in 1978 and subsequently fell to 18% over the ensuing decade (6). In recent years, resistance to penicillin, macrolides, and chloramphenicol has also become increasingly common among pneumococci (6, 9), and multidrug-resistant clones have been identified in regional outbreaks (11, 14). Reflected in our collection of strains were pneumococci (as well as viridans group and group A, B, C, and G streptococci) resistant to both tetracycline and minocycline, all but one of which were inhibited by each of the glycylcyclines at 1 μg/ml or less.

New antimicrobial agents with activity against multidrug-resistant enterococci are clearly needed (3). Representative strains of *E. faecium* isolates resistant to all of the currently available antimicrobial agents tested are included in Table 1. CL 329,998 and CL 331,002 were the only agents which showed significant activity against such isolates, inhibiting 90% of vancomycin-resistant *E. faecium* at concentrations of ≤0.25 and ≤0.5 μg/ml, respectively. As would be anticipated for tetracyclines, the new glycylcyclines demonstrated only bacteriostatic activities against the enterococcal strains we tested. However, inhibitory activity alone might be sufficient in the treatment of infections other than endocarditis or meningitis caused by such organisms.

The two glycylcyclines which we examined demonstrated activity against a wide range of gram-positive bacteria, including multidrug-resistant organisms. This activity extended to both tetracycline- and minocycline-resistant isolates of various species. The two compounds have also shown efficacy against strains of *S. aureus* (including a minocycline-resistant strain) in a model of acute lethal infection in mice (16). These agents appear to be worthy candidates for further in vitro and in vivo investigation.

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