ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1985, p. 357–362
0066-4804/85/030357-06$02.00/0

Vol. 27, No. 3

In Vitro Activity and Mechanism of Action of A21978C_1, a Novel Cyclic Lipopeptide Antibiotic

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Received 23 August 1984/Accepted 18 December 1984

The in vitro activity of A21978C_1, a novel cyclic polypeptide antibiotic, was compared with those of vancomycin, teichomycin, and several β-lactam antibiotics against gram-positive bacteria. The new drug was at least as active as vancomycin against all species of streptococci and staphylococci tested, including methicillin-resistant *Staphylococcus aureus* and penicillin-resistant pneumococci. Activity of the drug was found to be strongly correlated with the calcium concentration in test media. Against enterococci, A21978C_1 was bactericidal at concentrations near the MIC (MIC for 100% of the strains, 2 μg/ml), but combining that drug with gentamicin resulted in bactericidal synergism by time-kill methods. Studies were undertaken to examine the mechanism of action of the drug. A21978C_1 did not interact with penicillin-binding proteins of bacterial cell membranes. No direct effect of the drug on the synthesis of DNA, RNA, or protein by a susceptible strain of *Streptococcus faecalis* could be demonstrated. However, A21978C_1 inhibited peptidoglycan synthesis in early-log-phase cultures of both *Streptococcus faecalis* and *Staphylococcus aureus*.

A21978C_1, a new cyclic polypeptide antibiotic containing a fatty acid moiety, is representative of a novel class of acidic peptide antimicrobial agents derived from *Streptomyces roseosporus* (M. Debono, M. Barnhart, C. B. Carrell, J. A. Hoffman, and R. L. Hamill, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no. 68, 1980). The chemical structure of this antibiotic is shown in Fig. 1 (M. Debono, M. Barnhart, J. A. Hoffman, J. Occolowitz, B. J. Abbott, D. S. Fukida, R. L. Hamill, K. Biemann, and W. C. Herlihey; manuscript in preparation). Preliminary data indicate that the new drug is active against a broad range of gram-positive bacteria but shows little activity against gram-negative organisms (F. T. Counter, P. W. Ensminger, and L. C. Howard, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 20th, New Orleans, La., abstr. no. 69, 1980).

The present study characterizes the in vitro activity of A21978C_1 against various gram-positive bacteria, including strains which display nonenzymatic resistance to β-lactam antibiotics. The activity of the drug was compared with those of other peptide antibiotics, vancomycin and teichomycin (16), and several β-lactam antibiotics. As a result of finding unexpectedly poor activity of A21978C_1 in several commercial broth media, further studies were undertaken to examine medium dependence of the antimicrobial activity of the drug. Possible mechanisms of drug action were explored by examining its effects on the bacterial synthesis of DNA, RNA, protein, and peptidoglycan.

(This work was presented in part at the 23rd Interscience Conference on Antimicrobial Agents and Chemotherapy [G. M. Eliopoulos, C. Thauvin, and R. C. Moellering, Jr., Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 541, 1983].)

MATERIALS AND METHODS

Organisms. Bacterial strains used in this study were clinical isolates collected at the Massachusetts General Hospital, Boston, Mass., with the exception of penicillin-resistant pneumococci and viridans streptococci which had been collected in South Africa (4).

Antimicrobial agents and chemicals. Standard antimicrobial reference powders were obtained from the following sources: A21978C_1, Eli Lilly & Co., Indianapolis, Ind.; teichomycin, Dow Pharmaceuticals, Indianapolis, Ind.; amoxicillin, Beecham Laboratories, Bristol, Tenn.; ampicillin and cloxacillin, Bristol Laboratories, Syracuse, N.Y.; piperacillin, Lederle Piperacillin, Inc., Carolina, P.R.; imipenem, Merck, Sharpe & Dohme Research Laboratories, Rahway, N.J. Vancomycin sulfate was obtained from Eli Lilly. Antibiotic solutions were freshly prepared on the day of use. Other chemicals were purchased from commercial sources. Radiochemicals were purchased from New England Nuclear Corp., Boston, Mass.

Susceptibility studies. MICs were determined by an agar dilution technique (17) with Mueller-Hinton agar (BBL Microbiology Systems, Cockeysville, Md.). This medium was supplemented with 5% defibrinated sheep blood when nonenterococcal streptococci were tested. Inocula of ca. 10^4 CFU were prepared by appropriate dilutions of overnight cultures of test organisms in fresh Mueller-Hinton broth (BBL) and applied with a 32-prong inoculator. Plates were examined for growth after 18 to 20 h of incubation at 37°C.

Selected strains were also tested by a tube dilution method employing several broths: Todd-Hewitt broth (BBL) Trypticase soy broth (BBL), brain heart infusion (Difco Laboratories, Detroit, Mich.), and dextrose phosphate broth (DPB; GIBCO Diagnostics, Madison, Wis.). Final inocula of ca. 5×10^5 to 1×10^6 CFU/ml were prepared by diluting overnight cultures in fresh broth. MICs were determined by visual inspection for lack of turbidity after 18 to 20 h of incubation at 37°C. Samples of 0.01 ml from clear tubes were transferred to antibiotic-free plates. MBCs, as defined by a 99.9% reduction in CFU relative to the inoculum, were determined by the method of Pearson et al. (13), assuming a 5% pipetting error.

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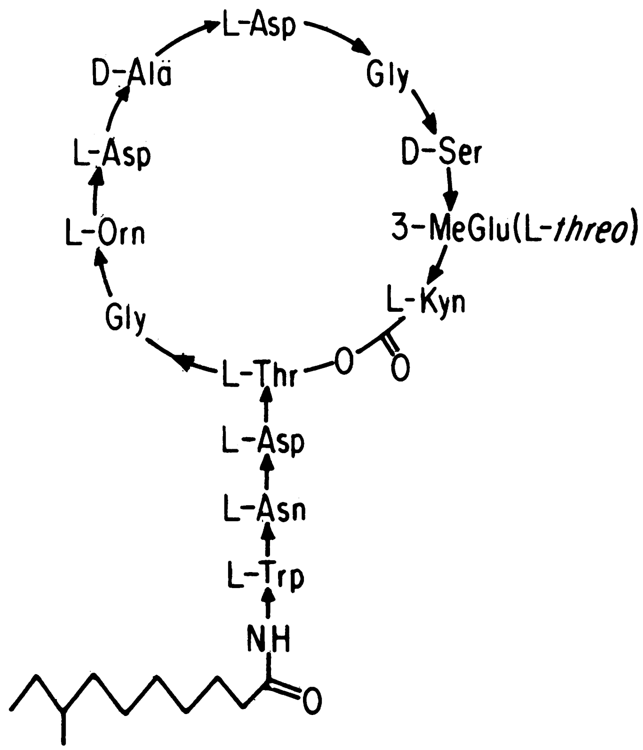

FIG. 1. Chemical structure of A21978C$_1$.

Effect of medium supplements. The effect of various organic and inorganic cations on the in vitro activity of A21978C$_1$ was examined by supplementing standard broths with these cations. Inorganic cations as the chloride salts were obtained from various commercial sources. Organic cationic compounds were purchased from Sigma Chemical Co., St. Louis, Mo. Susceptibility studies were performed as above, except that microtiter trays were employed.

Synergism studies. The bactericidal activity of A21978C$_1$ alone and in combination with gentamicin against enterococcal isolates was determined by time-kill methods as previously described (5). These studies were performed in DPB, with or without calcium supplementation (50 mg/liter). Synergism was defined as a 100-fold or greater reduction in CFU by the combination relative to that observed with A21978C$_1$ alone after 24 h of incubation.

Stability of A21978C$_1$. An agar well diffusion microbiological assay (14) for A21978C$_1$ was developed by using a strain of *Staphylococcus epidermidis* as the test organism. To test the stability of the drug, A21978C$_1$ was added to distilled water or various broths at concentrations of 100 to 200 μg/ml. Solutions were incubated at 37°C, and samples were withdrawn at specified times to determine residual concentrations of the antibiotic. All samples were tested in triplicate. To determine whether A21978C$_1$ was degraded by bacteria, flasks containing A21978C$_1$ at a concentration of 100 μg/ml in 19 ml of DPB were inoculated with 1.0 ml of an overnight culture of *Streptococcus faecalis*. This concentration of antibiotic permitted dense bacterial growth after 24 h of incubation. Samples of 1.0 ml were withdrawn at specified times, and the bacteria were removed by centrifugation at 10,000 × *g* for 10 min. Residual concentrations of A21978C$_1$ in the supernatant were determind by microbiological assay.

Penicillin-binding protein studies. Possible interaction of A21978C$_1$ with penicillin-binding proteins was studied by using cell membranes prepared from a strain of *Streptococcus faecalis*. Residual binding of [^{3}H]penicillin after preincubation of membranes with A21978C$_1$ was determined by methods previously described in detail (6).

Effects on synthesis of macromolecules. The effect of A21978C$_1$ on DNA, RNA, and protein synthesis in *Streptococcus faecalis* was studied by using the methods of Crumplin and Smith (3). In brief, log-phase cells were grown in DBP (with 50 mg of calcium per liter) containing [^{3}H]thymidine, [^{3}H]uridine, or [^{3}H]leucine (0.1 μCi/ml, final concentration). A21978C$_1$ at a concentration of 4 μg/ml was added to two sets of tubes, while a third set, without antibiotic, served as a control. Samples were withdrawn at intervals over 120 min. Trichloroacetic acid-precipitable material from samples labeled with [^{3}H]thymidine and [^{3}H]leucine was collected over Whatman GF/C filters, washed, and counted in a Beckman scintillation counter to measure DNA and protein synthesis. RNA synthesis was calculated by subtracting incorporation of [^{3}H]uridine into alkali-stable material from total [^{3}H]uridine incorporation into trichloroacetic acid-precipitable material. Colony counts and total protein content (10) were determined at each sampling period in parallel specimens, prepared as above except that the radiochemicals were omitted. These data were used to normalize the measured uptake of labeled precursors for changes in cell mass or the number of viable cells or both.

To assess more directly the effect of A21978C$_1$ on ribosomal protein synthesis in a cell-free system, the method of Nirenberg (12) was used, with minor modifications as described by Farber et al. (7).

Peptidoglycan synthesis in *Streptococcus faecalis* was studied by using the method described by Lugtenberg and deHaan (11). The incorporation of [^{14}C]alanine into trichloroacetic acid-precipitable peptidoglycan was carried out in CWSM-I medium (11) modified by the substitution of lysine for diaminopimelic acid and by the addition of calcium chloride (50 mg/liter). Chloramphenicol (50 μg/ml) was added to inhibit protein synthesis. A21978C$_1$ was added at a concentration of 100 μg/ml, and the net incorporation of radioactivity into trichloroacetic acid-precipitable material was compared with amounts detected in specimens obtained from a control flask which lacked the antibiotic.

Calcium content of media. Calcium concentration in various media was determined by low-temperature flame atomic absorption spectrophotometry. In this procedure, dilutions of a sample are atomized into an air-acetylene flame through which light of a characteristic wavelength for calcium is directed (Atomic Absorption Spectrophotometer model 503, The Perkin Elmer Corp., Norwalk, Conn.). The decrease in signal is related to the concentration of calcium in the sample (1, 2).

RESULTS

Agar dilution studies. Results of agar dilution susceptibility studies utilizing Mueller-Hinton agar are shown in Table 1. A21978C$_1$ was at least as active as vancomycin against all species of streptococci and staphylococci tested. The new drug demonstrated marked activity against methicillin-resistant strains of *Staphylococcus aureus* and against penicillin-resistant pneumococci and viridans streptococci. Against *Listeria monocytogenes*, A21978C$_1$ was significantly less active than vancomycin, teichomycin, or any of the β-lactams tested. The activity of A21978C$_1$ against enterococci, like that of vancomycin, was only modestly influenced by inoculum size (Table 2).

TABLE 1. Comparative in vitro activity of A21978C_1 against gram-positive bacteria

Strain (no.)	Antibiotic	MIC range (μg/ml)	MIC (μg/ml) for % of strains:	
			50	90
Streptococcus faecalis (87)	A21978C_1	0.125–2	0.5	1.0
	Vancomycin	0.25–8	1.0	2
	Teichomycin	≤0.06–0.5	0.25	0.5
	Ampicillin	0.25–2	1.0	1.0
	Amoxicillin	0.125–1.0	0.5	1.0
	Piperacillin	2–8	2	4
	Imipenem	0.25–4	1.0	4
Streptococcus faecium (10)	A21978C_1	1.0–2	2	2
	Vancomycin	1.0–2	1	2
	Teichomycin	0.125–0.5	0.25	0.5
	Ampicillin	0.5–8	2	4
	Amoxicillin	0.5–4	1	2
	Piperacillin	16–128	32	128
	Imipenem	4–32	4	32
Staphylococcus aureus (20) (methicillin susceptible)	A21978C_1	0.25–0.5	0.25	0.5
	Vancomycin	0.25–1.0	0.25	0.5
	Teichomycin	0.125–0.5	0.25	0.5
	Amoxicillin	1.0–32	4	16
	Piperacillin	4–≥256	64	≥256
	Cloxacillin	0.125–1.0	0.25	0.5
	Imipenem	≤0.06	≤0.06	≤0.06
Staphylococcus aureus (10) (methicillin resistant)	A21978C_1	0.125–0.5	0.25	0.5
	Vancomycin	0.5–2	1.0	1.0
	Teichomycin	0.125–0.5	0.25	0.25
	Amoxicillin	64–≥256	64	≥256
	Imipenem	0.5–16	16	16
Staphylococcus epidermidis (10)	A21978C_1	0.125–0.5	0.5	0.5
	Vancomycin	1.0–2	2	2
	Teichomycin	0.125–2	0.5	2
	Ampicillin	2–64	16	64
	Amoxicillin	2–64	32	64
	Imipenem	≤0.06–32	1.0	16
Listeria monocytogenes (10)	A21978C_1	4–16	8	16
	Vancomycin	0.5–1.0	1.0	1.0
	Teichomycin	0.25–0.5	0.5	0.5
	Ampicillin	0.125–0.5	0.25	0.25
	Amoxicillin	≤0.06–0.25	0.125	0.25
	Piperacillin	0.125–2	1.0	2
	Imipenem	≤0.06–0.25	0.125	0.25
Streptococcus pyogenes (10)	A21978C_1	≤0.06	≤0.06	≤0.06
	Vancomycin	0,25–0.5	0.25	0.5
	Teichomycin	≤0.06	≤0.06	≤0.06
	Ampicillin	≤0.06	≤0.06	≤0.06
	Amoxicillin	≤0.06	≤0.06	≤0.06
	Piperacillin	≤0.06	≤0.06	≤0.06
	Imipenem	≤0.06	≤0.06	≤0.06
Streptococcus agalactiae (10)	A21978C_1	0.25–0.5	0.25	0.25
	Vancomycin	0.25–0.5	0.5	0.5
	Teichomycin	≤0.06–0.125	0.125	0.125
	Ampicillin	0.125	0.125	0.125
	Amoxicillin	≤0.06–0.125	≤0.06	0.125
	Piperacillin	0.125–2	0.125	0.25
	Imipenem	≤0.06	≤0.06	≤0.06
Group G streptococci (10)	A21978C_1	≤0.06	≤0.06	≤0.06
	Vancomycin	0.25–0.5	0.5	0.5
	Teichomycin	≤0.06–0.125	≤0.06	0.125
	Ampicillin	≤0.06	≤0.06	≤0.06
	Amoxicillin	≤0.06	≤0.06	≤0.06
	Piperacillin	≤0.06–0.25	0.125	0.125
	Imipenem	≤0.06	≤0.06	≤0.06

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TABLE 1—*Continued*

Strain (no.)	Antibiotic	MIC range (μg/ml)	MIC (μg/ml) for % of strains: 50	90
Viridans streptococci (9) (penicillin susceptible)	$A21978C_1$	≤0.06–4		
	Vancomycin	0.25–1.0		
	Teichomycin	≤0.06–0.25		
	Ampicillin	≤0.06–0.25		
	Amoxicillin	≤0.06–0.125		
	Piperacillin	≤0.06–2		
	Imipenem	≤0.06		
Viridans streptococci (10) (penicillin resistant)	$A21978C_1$	0.125–2	0.25	0.5
	Vancomycin	0.25–1.0	0.5	0.5
	Teichomycin	≤0.06–0.125	≤0.06	≤0.06
	Ampicillin	0.5–16	1.0	8
	Amoxicillin	0.5–4	1.0	4
	Piperacillin	0.5–16	4	16
	Imipenem	≤0.06–1.0	0.25	1.0
Streptococcus pneumoniae (7) (penicillin resistant)	$A21978C_1$	≤0.06–0.125		
	Vancomycin	≤0.06–0.5		
	Teichomycin	≤0.06–0.125		
	Ampicillin	≤0.06–4		
	Amoxicillin	≤0.06–2		
	Piperacillin	0.25–16		
	Imipenem	≤0.06–0.5		

Broth dilution studies. When the susceptibility of enterococci to $A21978C_1$ was examined in DPB, the results were unexpectedly poor, with MICs of 125 to 250 μg/ml. In other commercially available broths, $A21978C_1$ was 10- to 100-fold less active than in Mueller-Hinton agar. The activity of $A21978C_1$ against *Streptococcus faecalis* was highly correlated with the calcium content of the test medium ($r = -0.98$) (Fig. 2). Supplementation of commercial broths with physiological concentrations of calcium resulted in activity which was comparable to that determined on Mueller-Hinton agar (calcium content, 52 mg/liter; mean MIC, 2 μg/ml). A similar dependence of activity on calcium concentration was also seen with *Staphylococcus aureus* and group B streptococci.

The effect of other inorganic and organic cations on the activity of $A21978C_1$ against a representative strain of *Streptococcus faecalis* is shown in Table 3. No supplement tested except calcium significantly affected the activity of the antimicrobial agent. Activities of penicillin, cycloserine, vancomycin, or bacitracin against this strain were not influenced by the calcium concentration.

The lipopeptide was bactericidal at concentrations within one dilution of the MIC against *Streptococcus faecalis*, viridans and group B streptococci, and *Staphylococcus aureus* in several commercial broths. When bactericidal

TABLE 2. Effect of inoculum size on the activities of $A21978C_1$ and vancomycin against enterococci

Strain (no.)	Antibiotic	Inoculum (CFU)	MIC (μg/ml) for % of strains: 50	90
Streptococcus faecalis (20)	$A21978C_1$	10^5	2	2
		10^3	0.5	1.0
		10^2	0.5	1.0
	Vancomycin	10^5	1.0	4
		10^3	1.0	2
		10^2	1.0	2
Streptococcus faecium (10)	$A21978C_1$	10^5	4	4
		10^3	4	4
		10^2	2	4
	Vancomycin	10^5	1.0	4
		10^3	1.0	2
		10^2	0.5	1.0

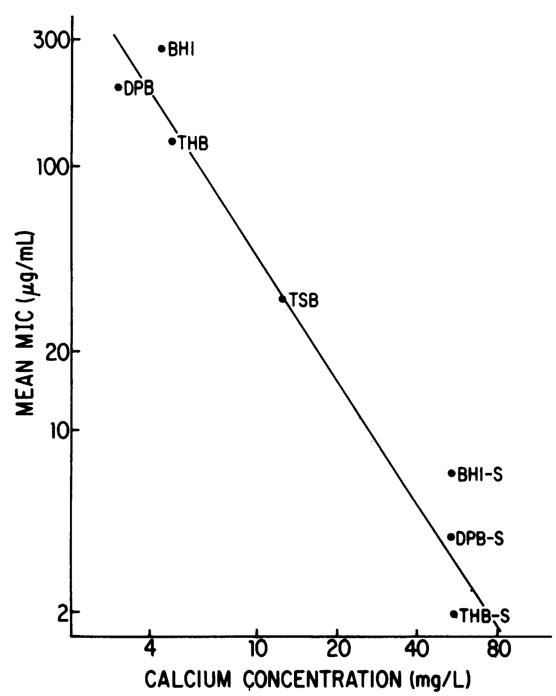

FIG. 2. Effect of calcium concentration in various media on the activity of $A21978C_1$. Each point represents the geometric mean MIC of the antibiotic against six strains of *Streptococcus faecalis*. BHI, Brain heart infusion; THB, Todd-Hewitt broth; TSB, Trypticase soy broth. DPB-S, THB-S, and BHI-S, the above broths supplemented with calcium to a concentration of 50 mg/liter.

activity was determined in calcium-supplemented broths, modal MBCs of A21978C1 were twice the modal MICs against the streptococci, whereas model MBCs against methicillin-susceptible and -resistant strains of *Staphylococcus aureus* (8 to 16 μg/ml) were severalfold greater than the modal MIC (1 μg/ml).

Synergism studies. Combinations of A21978C1 (10 μg/ml) with gentamicin (5 μg/ml) were tested against five strains of *Streptococcus faecalis*. The combination demonstrated bactericidal synergism against all strains. The magnitude of increased killing by the combination relative to killing by A21978C1 alone at 24 h ranged from 2 to 5 $\log_{10}$ units.

Antibiotic stability. The poor activity of A21978C1 in commercial broths prompted an investigation of its stability in liquid media. The compound retained at least 80% of its bioassayable activity when incubated up to 48 h at 37°C in distilled water, DPB, Mueller-Hinton broth, and brain heart infusion. Twenty-four hours after exposure of subinhibitory concentrations of the drug to growing cultures of *Streptococcus faecalis*, 92% of the initial bioassayable activity of A21978C1 remained in the culture supernatant.

Mechanism of action. Competition studies utilizing cell membranes prepared from *Streptococcus faecalis* revealed no binding of A21978C1 to penicillin-binding proteins of this organism. Studies of macromolecular synthesis showed no direct effect of A21978C1 on the synthesis of DNA, RNA, or protein. After 130 and 100 min, respectively, net incorporation of [^{3}H]thymidine and [^{3}H]uridine (alkali-labile fraction) in the presence of A21978C1 at an inhibitory concentration (4 μg/ml) differed by less than 5% from levels measured in the absence of antibiotic. Net uptake of radiolabeled precursors (normalized for protein content of the incubation mixture) in the presence and absence, respectively, of A21978C1 was 1,069 and 1,122 cpm (DNA) and 4,134 and 4,313 cpm (RNA).

TABLE 3. Effect of supplemental cations on the activity of A21978C1 against *Streptococcus faecalis* 1310 in DPB

Cation	Concn added (mM)	MIC (μg/ml)
None		125
Na^+	1.0	125
	10	63
	100	125
K^+	1.0	63
	4.0	125
Ca^{2+}	0.25	63
	0.63	16
	1.25	4
	2.5	4
Ba^{2+}	1.0	250
Zn^{2+}	1.0	63
Mg^{2+}	1.0	125
Putrescine^{2+}	1.25	125
	10	125
Spermidine^{3+}	1.25	63
	10	63
Spermine^{4+}	1.25	63
	10	125

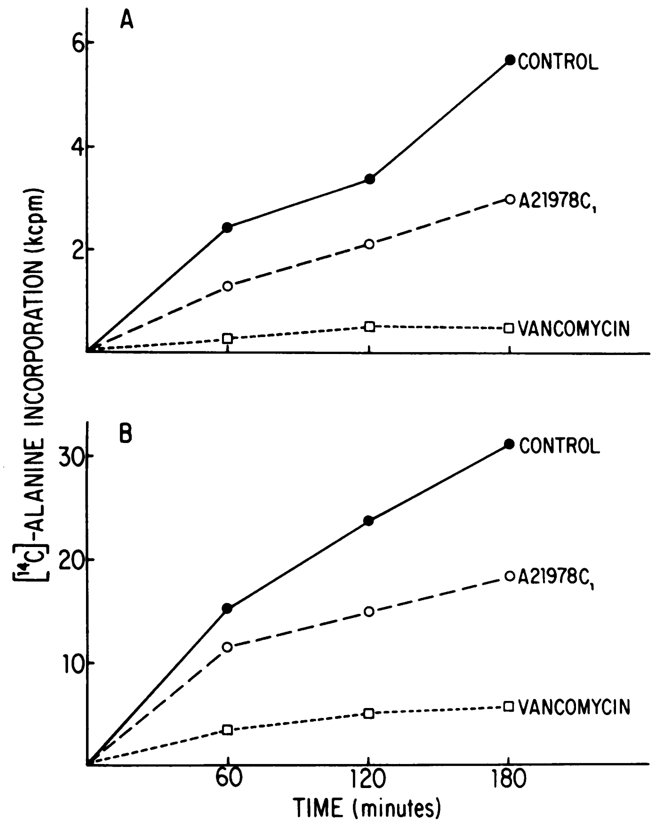

FIG. 3. Effect of A21978C1 and vancomycin on incorporation of [^{14}C]alanine into peptidoglycan by strains of *Streptococcus faecalis* (A) and *Staphylococcus aureus* (B). Each antibiotic was added at a concentration of 100 μg/ml.

Concentrations of the drug up to 100 μg/ml caused less than a 10% decrease in polyuridylic acid-directed synthesis of polyphenylalanine in the cell-free system.

The effect of A21978C1 on peptidoglycan synthesis was dependent upon the growth phase of the organisms when exposed to the antibiotic. When cultures of either *Streptococcus faecalis* or *Staphylococcus aureus* were used in early log phase, addition of A21978C1 (100 μg/ml) resulted in ca. 50% inhibition of [^{14}C]alanine incorporation after 180 min of incubation (Fig. 3). In contrast, peptidoglycan synthesis in late-log-phase or stationary-phase cultures of *Streptococcus faecalis* (8% inhibition) and *Staphylococcus aureus* (20% inhibition) was less affected by the antibiotic. In log-phase cultures of the *Streptococcus faecalis* strain, A21978C1 caused a 25% reduction in [^{14}C]alanine incorporation when added at a concentration of 50 μg/ml. No inhibition of peptidoglycan synthesis was seen when the drug was added in concentrations of 5 and 10 μg/ml. At the latter concentration, vancomycin caused a 49% inhibition of [^{14}C]alanine incorporation.

DISCUSSION

By standard agar dilution techniques, the new cyclic lipopeptide A21978C1 demonstrated excellent activity against a broad range of gram-positive bacteria, including methicillin-resistant strains of *Staphylococcus aureus* and *Staphylococcus epidermidis* and penicillin-resistant pneumococci and viridans streptococci. MICs of A21978C1 were comparable to those of vancomycin and teichomycin. Notably, the new antibiotic was markedly less active than vancomycin or teichomycin against *L. monocytogenes*. The fact that $A21978C_1$ was bactericidal against enterococci at concentrations near the MIC may be of particular significance, since treatment of serious enterococcal infections with currently available antibiotics generally requires the use of potentially toxic regimens that combine a cell wall-active drug with an aminoglycoside (15).

The observation that combinations of $A21978C_1$ with gentamicin resulted in bactericidal synergism against enterococci suggested that the polypeptide might act at the level of the cell wall, since the ability to produce synergistic killing when combined with an aminoglycoside is characteristic of cell wall-active antibiotics (9). Studies of peptidoglycan synthesis in our whole cell system demonstrated that $A21978C_1$ at a concentration of 100 μg/ml inhibited peptidoglycan synthesis in *Streptococcus faecalis* and *Staphylococcus aureus*. The fact that $A21978C_1$ at concentrations of 5 to 10 μg/ml did not result in decreased [^{14}C]alanine incorporation in the experimental system used in this study cannot be taken as evidence against inhibition of peptidoglycan synthesis as a primary mechanism of antibiotic action. Because of the need to add chloramphenicol to the test medium to block incorporation of the radiolabeled amino acid into protein, the actual MIC of $A21978C_1$ in this medium cannot be determined. Other experiments revealed no evidence that the new antibiotic directly inhibited synthesis of DNA, RNA, or protein in *Streptococcus faecalis*. Nevertheless, the fact that inhibition of peptidoglycan synthesis by $A21978C_1$ was incomplete and less than that resulting from exposure to vancomycin under identical conditions (Fig. 3) suggests that there may be additional sites of antimicrobial action. Our results are consistent with those of Allen et al., who have reported that the antibiotic LY146032, an analog of $A21978C_1$, blocks incorporation of radiolabeled amino acids into peptidoglycan of *Staphylococcus aureus* and *Bacillus megaterium* (N. Allen, W. Alborn, Jr., J. Hobbs, Jr., and H. Percifield, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 24th, Washington, D.C., abstr. no. 1081, 1984).

Initial attempts to determine activity of $A21978C_1$ in various broth media led to unexpectedly poor results which could not be explained by instability of the drug in liquid media or by inactivation of the antibiotic by growing cultures of bacteria. This discrepancy between activity of $A21978C_1$ in (calcium-poor) commercial broth media and the activity of the drug in agar media was resolved with the demonstration of a strong correlation between antibacterial activity and calcium content of the growth medium. Supplementation of media with other inorganic or organic cations resulted in negligible effects on the activity of the drug.

The reasons for this unusual dependence of the activity of the drug on calcium concentrations in growth media are at present unclear. The cation may be required either for penetration of $A21978C_1$ into the bacterial cell or to facilitate a direct antimicrobial effect of the drug at the level of peptidoglycan synthesis or elsewhere. Alternatively, the polypeptide may interfere with the active extrusion of calcium from bacterial cells (8), resulting in damage due to intracellular accumulation of the cation.

The demonstrated in vitro activity of $A21978C_1$ against gram-positive bacteria, many of which cause infections which are difficult to treat with currently available agents, would alone appear to warrant further studies with the polypeptide or with other members of this novel class of antibiotics. In addition, the unusual dependence of the activity of the drug on the calcium concentration in the media suggests that investigations to ascertain more precisely the mode of action of this antibiotic would be of particular importance.

ACKNOWLEDGMENTS

We thank N. Allen and D. Preston of Eli Lilly & Co. for useful discussions and advice.

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