ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1987, p. 1014–1016
0066-4804/87/071014-03$02.00/0

Vol. 31, No. 7

Bactericidal Activity of Deptomycin (LY146032) Compared with Those of Ciprofloxacin, Vancomycin, and Ampicillin against Enterococci as Determined by Kill-Kinetic Studies

CHARLES W. STRATTON,* CONNIE LIU, HILDA B. RATNER, AND LYNDELL S. WEEKS
Department of Pathology, Vanderbilt University School of Medicine, Nashville, Tennessee 37232

Received 16 January 1987/Accepted 8 April 1987

This study used kill-kinetic methods to provide data on the bactericidal activity of subinhibitory (1/2× MIC), inhibitory (1× MIC), and suprainhibitory (4×, 6×, and 8× MIC) concentrations of deptomycin (LY146032) against strains of enterococci compared with those of ciprofloxacin, vancomycin, and ampicillin. Deptomycin was the most active agent tested, as determined by broth microdilution methods, with all strains being inhibited at concentrations ≤2 μg/ml. The kill-kinetic studies demonstrated that deptomycin had greater activity at all concentrations tested than the other cell wall-active agents; regrowth was seen, however, at lower concentrations. At higher concentrations (6× and 8× MIC), all agents tested demonstrated the same or less bactericidal activity than at 4× MIC, presumably due to the Eagle effect. Nevertheless, these results suggest that further evaluation of deptomycin as a therapeutic agent for serious enterococcal infections is warranted.

Deptomycin (LY146032) is a cyclic polypeptide which by standard broth dilution techniques has been shown to be bactericidal against enterococci at concentrations near the MIC (4, 6). This suggests that deptomycin may be useful clinically as a single agent against serious enterococcal infections, including endocarditis. This study used kill-kinetic methods to provide data on the bactericidal activity of subinhibitory (1/2× MIC), inhibitory (1× MIC), and suprainhibitory (4×, 6×, and 8× MIC) concentrations of deptomycin against strains of enterococci compared with those of ciprofloxacin, vancomycin, and ampicillin.

MATERIALS AND METHODS

Microorganisms. Ten clinical isolates of enterococci and *Streptococcus faecalis* ATCC 29212 were studied. These isolates were selected from more than 200 blood culture isolates of enterococci for which the MICs of vancomycin, ampicillin, and gentamicin had been determined previously. Selection was done to provide a wide range of MICs of the three agents previously tested. Identification was performed by established methods (5), which included the ability to grow in medium containing 40% bile, to hydrolyze esculin, and to grow in broth containing 6.5% sodium chloride. All strains were identified as *S. faecalis*.

Antimicrobial agents. Standard antimicrobial reference powders and their sources were as follows: deptomycin and vancomycin, Eli Lilly & Co., Indianapolis, Ind.; ciprofloxacin, Miles Pharmaceuticals, West Haven, Conn.; and ampicillin, Bristol Laboratories, Syracuse, N.Y. Antimicrobial agent stock solutions were prepared as specified by the manufacturers and stored at −70°C until use. Final concentrations were prepared on the day they were used.

Media. Mueller-Hinton broth (BBL Microbiology Systems, Cockeysville, Md.) was used in both the broth microdilution method and the kill-kinetic method. The broth was supplemented with physiologic concentrations of calcium and magnesium to achieve a final concentration of 50 mg of Ca^{2+} and 25 mg of Mg^{2+} per liter of medium (16).

Inoculum. Portions of five or more colonies of a plate culture were inoculated into Trypticase soy broth (BBL) and incubated. The turbidity of the logarithmic-phase culture was adjusted to a 0.5 McFarland standard with Trypticase soy broth. Further dilutions were made as determined by the specific susceptibility test method with cation-supplemented Mueller-Hinton broth. The inoculum used was carefully controlled, and the final concentration was confirmed by a modified surface colony count method (14). For this method, 10-fold dilutions were made in physiologic saline, and a calibrated micropipetter was used to deliver five 0.05-ml portions from each dilution onto a sheep blood agar plate. These plates were incubated for 24 h, colonies were counted and averaged, and the final inoculum was calculated.

Broth microdilution method. This method was done by standard methods (16) with cation-supplemented Mueller-Hinton broth. Serial twofold dilutions (ranging from 0.0625 to 32 μg/ml) were prepared in broth and used to test each isolate. The final inoculum was 5×10^4 CFU per well and was confirmed each time by the modified surface colony count method as described above. A micropipetter that delivered 0.05 ml was used to inoculate each well of the microdilution plate (Dynatech Laboratories, Inc., Alexandria, Va). The final volume in each well was 0.1 ml. After 24 h of incubation at 35°C, the plates were removed and read with a microtiter mirror. The MIC was defined as the lowest concentration of the antimicrobial agent that inhibited growth of the test organism. MICs were determined in duplicate on at least two separate occasions. *S. faecalis* ATCC 29212 was used each time for quality assurance.

Kill-kinetic studies. Kill-kinetic studies were done in duplicate on separate occasions essentially as described by Krogstad and Moellering (8) and Chalkley and Koornhof (1). All kill-kinetic studies were done with a final volume of 30 ml of broth in glass bottles. Borosilicate glass bottles without medium were kindly supplied by Johnston Laboratories (Baltimore, Md.). Cation-supplemented Mueller-Hinton broth was used as the medium. Both the antimicrobial agent to be tested and the inoculum were added so as to maintain a final volume of 30 ml. The inoculum added was calculated

* Corresponding author.

TABLE 1. MICs of selected antimicrobial agents for 11 enterococcal isolates by the broth microdilution method

Antibiotic	MIC (μg/ml)[a]		
	Range	50%	90%
Deptomycin	0.0625–2	1	2
Ciprofloxacin	1–4	2	4
Vancomycin	1–4	2	4
Ampicillin	2–32	4	16

[a] Data for four determinations. 50% and 90%, MIC for 50 and 90% of strains tested, respectively.

to achieve a final concentration of 10^5 CFU/ml. Each isolate was tested in kill-kinetic studies to establish the effect of subinhibitory (1/2× MIC), inhibitory (1× MIC), and suprainhibitory (4×, 6×, and 8× MIC) concentrations of the antibiotics. The concentration of each antimicrobial agent against each microorganism was determined by the MIC for that specific combination. Bottles were incubated on a shaker (Johnston Laboratories, Baltimore, Md.) at 35°C for 24 h, which allowed growth in the control bottles to reach a level of 10^9 to 10^{10} CFU/ml. Comparisons of bottles which were shaken versus bottle which were not shaken demonstrated that the regrowth seen at lower concentrations did not result from organisms washed back into the broth after having escaped antibiotic exposure on the walls of the flask. Bottles were subcultured for colony counts at 0, 4, 8, 12, 16, 20, and 24 h of incubation. Colony counts were performed by removing 0.5 ml of the broth with a syringe and making 10^{-1}, 10^{-2}, 10^{-3}, and 10^{-4} dilutions in sterile physiologic saline. A 0.02-ml portion of each dilution was plated onto sheep blood agar plates and incubated for 24 h. The number of colonies was counted and used to determine the number of viable CFU at each sampling time. Kill-kinetic curves were then plotted showing time versus CFU per milliliter of broth. The mean percent inoculum killed by specific concentrations at various times was then determined. Each strain was studied at least twice for all antibiotics.

RESULTS

Broth microdilution method. The results of the broth microdilution susceptibility tests are shown in Table 1. Deptomycin was the most active agent tested, with all strains being inhibited at concentrations of ≤2 μg/ml. Vancomycin and ciprofloxacin were quite active, inhibiting all strains at concentrations of ≤4 μg/ml.

Kill-kinetic studies. The bactericidal effect of each antibiotic at subinhibitory concentrations (1/2× MIC) was such that no agent killed even a tenth of the microorganism tested.

Table 2 indicates the effect of each antibiotic at inhibitory concentrations (1× MIC). No agent tested resulted in bactericidal activity (3 $\log_{10}$ killing). Ciprofloxacin had killed 97.7% of the final inoculum at 12 h, followed by slight regrowth. Deptomycin had killed 88.0% at 12 h, followed by moderate regrowth. Ampicillin demonstrated a steady increase in killing over time, killing 80.6% at 24 h. Vancomycin had killed 45.1% of the final inoculum at 24 h. Table 3 indicates the bactericidal effect of each antibiotic at 4× MIC. Overall, no agent tested resulted in bactericidal activity (≥3 $\log_{10}$ killing), although both deptomycin and ciprofloxacin showed ≥99% killing at 24 h. Vancomycin was the least active, killing only 69.5% at 24 h. Low MICs for individual isolates were most apt to give 3 $\log_{10}$ killing of the initial inoculum at 24 h. At higher concentrations (6× and 8× MIC), the bactericidal effect for isolates with low MICs usually was similar to that of 4× MIC and reflected a 3 $\log_{10}$ killing of the initial inoculum. For isolates with higher MICs, the bactericidal effect most often was less than that of 4× MIC. The concentrations used for these isolates were usually higher than would be achieved clinically.

TABLE 2. Inoculum killed at inhibitory (1 × MIC) concentrations of each antibiotic at various times for 11 enterococcal isolates

Time (h)	Mean % killing			
	Deptomycin	Ciprofloxacin	Vancomycin	Ampicillin
4	35.3	63.1	0[a]	49.9
8	78.7	95.3	1.8	69.7
12	88.0	97.7	20.9	62.5
24	61.5	90.3	45.1	80.6

[a] A zero indicates that the final CFU count was equal to or greater than the initial inoculum.

TABLE 3. Inoculum kill at suprainhibitory (4 × MIC) concentrations of each antibiotic at various times for 11 enterococcal isolates

Time (h)	Mean % killing			
	Deptomycin	Ciprofloxacin	Vancomycin	Ampicillin
4	78.8	82.3	6.8	62.7
8	94.7	98.3	15.6	76.3
12	97.6	99.0	35.9	83.0
24	99.2	99.4	69.5	95.7

DISCUSSION

Enterococci rank as the third most common cause of endocarditis behind viridans group streptococci and *Staphylococcus aureus*. This pathogen is isolated from 10 to 15% of patients with endocarditis (10–12, 20). Therapy for patients with enterococcal endocarditis is difficult because enterococci are tolerant to the bactericidal activity of cell wall-active agents such as ampicillin and vancomycin (7, 9). In addition, aminoglycoside resistance is a potential problem (13). Because optimal therapy for infective endocarditis depends on obtaining bactericidal activity in vivo (2, 22) the use of beta-lactam agents, vancomycin, or aminoglycosides alone is not feasible. Instead, combined therapy (a cell wall-active agent with an aminoglycoside) is routinely used for enterococcal endocarditis (17, 19, 20, 22).

Deptomycin is a recently developed antibiotic (4, 6) which belongs to a novel class of acidic lipopeptide agents (3). Deptomycin has demonstrated significant in vitro activity against enterococci, with bactericidal activity occurring near the MIC (4, 6). The antimicrobial properties of this new lipopeptide appear to result from its ability to inhibit cell wall peptidoglycan synthesis (N. Allen, W. Alborn, Jr., J. Hobbs, Jr., and H. Percifield, Program Abstr. 24th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 1081, 1984). Because other cell wall-active agents, including penicillins and vancomycin, are only bacteriostatic against enterococci (9), the bactericidal activity of deptomycin, a new member of the cell wall-active group of antibiotics, against enterococci became an important issue. Therefore, kill-kinetic studies were used to provide data on the bactericidal activity of subinhibitory (1/2× MIC), inhibitory (1× MIC), and suprainhibitory (4×, 6×, and 8× MIC) concentrations of

deptomycin against strains of enterococci compared with those of ciprofloxacin, vancomycin, and ampicillin. Both cell wall-active agents (deptomycin, vancomycin, and ampicillin) and a non-cell-wall-active agent (ciprofloxacin) were included in this study. The in vitro activity of the acidic lipopeptide agents (including deptomycin) demonstrates a striking dependence on the calcium concentration in the growth medium (3, 4, 6); accordingly, cation-supplemented Mueller-Hinton broth was used for all susceptibility tests.

Deptomycin was the most active agent tested, as determined by broth microdilution methods, with all strains being inhibited at concentrations of ≤ 2 μg/ml. Kill-kinetic studies demonstrated that deptomycin had greater activity at all concentrations tested than did the other cell wall-active agents tested; however, regrowth was frequently seen at lower concentrations. The bactericidal activity at 6× and 8× MIC was less for isolates with higher MICs than the activity at 4× MIC. Presumably, this is due to the Eagle effect. There are few data on achievable serum concentrations of deptomycin in humans. Studies with monkeys (Eli Lilly & Co., personal communication) suggest that the achievable serum concentrations of deptomycin in humans may not exceed the concentrations studied.

An animal model of enterococcal endocarditis has confirmed, at least in part, the bactericidal activity of deptomycin under physiologic conditions, with a 3 $\log_{10}$ reduction in bactericidal titers within cardiac vegetations (in comparison with controls) after only 5 days of therapy (4). Similar studies by others have indicated that more prolonged courses of deptomycin are equally effective in clearing enterococci from cardiac vegetations (A. M. F. Hansen, D. H. Holmes, D. A. Preston, and R. Pekarek, 24th ICAAC, abstr. no. 1079, 1984). However, it is still unclear despite these data whether deptomycin as a single agent would prove more effective than ampicillin alone in the therapy of enterococcal endocarditis in humans (C. Thauvin, G. M. Eliopoulos, S. Willey, and R. C. Moellering, Jr., 24th ICAAC, abstr. no. 338, 1984). Finally, these data do not address the influence of human serum on the bactericidal activity of antimicrobial agents against enterococci. Storch and Krogstad (18) have shown that antibiotic-induced lysis of enterococci is markedly decreased in human serum. Nevertheless, there are sufficient data on the bactericidal activity of deptomycin, including that obtained from this study, to warrant further evaluation of this drug as a therapeutic agent for serious enterococcal infections, including endocarditis. The availability of an additional non-beta-lactam cell wall-active agent with reasonable bactericidal activity against enterococci may become particularly important should beta-lactamase-producing strains (15) become a problem.

ACKNOWLEDGMENT

This study was supported by a grant from Lilly Research Laboratories, Indianapolis, Ind.

LITERATURE CITED

1. **Chalkley, L. J., and H. J. Koornhof.** 1985. Antimicrobial activity of ciprofloxacin against *Pseudomonas aeruginosa*, *Escherichia coli*, and *Staphylococcus aureus* determined by the killing curve method: antibiotic comparisons and synergistic interactions. Antimicrob. Agents Chemother. **26**:310–313.
2. **Drake, T. A., and M. A. Sande.** 1983. Studies of the chemotherapy of endocarditis: correlation of in vitro, animal model, and clinical studies. Rev. Infect. Dis. **5**(Suppl. 2):S345–S354.
3. **Eliopoulos, G. M., C. Thauvin, B. Gerson, and R. C. Moellering, Jr.** 1985. In vitro activity and mechanism of action of A21978C_1, a novel cyclic lipopeptide antibiotic. Antimicrob. Agents Chemother. **27**:357–362.
4. **Eliopoulos, G. M., S. Willey, E. Reiszner, P. G. Spitzer, G. Caputo, and R. C. Moellering, Jr.** 1986. In vitro and in vivo activity of LY146032, a new cyclic lipopeptide antibiotic. Antimicrob. Agents Chemother. **30**:532–535.
5. **Facklam, R. R., and R. B. Carey.** 1985. Streptococci and aerococci, p. 154–175. *In* E. H. Lennette, A. Balows, W. J. Hausler, Jr., and J. P. Truant (ed.), Manual of clinical microbiology, 4th ed. American Society for Microbiology, Washington, D.C.
6. **Fass, R. J., and V. L. Helsel.** 1986. In vitro activity of LY146032 against staphylococci, streptococci, and enterococci. Antimicrob. Agents Chemother. **30**:781–784.
7. **Herstein, J., J. L. Ryan, R. J. Mangi, T. P. Greco, and V. T. Andriole.** 1984. Optimal therapy for enterococcal endocarditis. Am. J. Med. **76**:186–191.
8. **Krogstad, D. J., and R. C. Moellering, Jr.** 1986. Antimicrobial combinations, p. 546. *In* V. Lorian (ed.), Antibiotics in laboratory medicine, 2nd ed. The Williams & Wilkins Co., Baltimore.
9. **Krogstad, D. J., and A. R. Parquette.** 1980. Defective killing of enterococci: a common property of antimicrobial agents acting on the cell wall. Antimicrob. Agents Chemother. **17**:965–968.
10. **Lowes, J. A., J. Hamer, G. Williams, E. Houarg, S. Tabaqchali, E. J. Shaw, I. M. Hill, and G. M. Rees.** 1980. Ten years of infective endocarditis at St. Bartholomew's Hospital: analysis of clinical features and treatment in relation to prognosis and mortality. Lancet **ii**:133–136.
11. **Malone, D. A., R. A. Wagner, J. P. Myers, and C. Watanakunakorn.** 1986. Enterococcal bacteremia in two large community teaching hospitals. Am. J. Med. **81**:601–606.
12. **Mandell, G. L., D. Kaye, M. E. Levison, and E. W. Hook.** 1970. Enterococcal endocarditis: an analysis of 38 patients observed at New York Hospital-Cornell Medical Center. Arch. Intern. Med. **125**:258–264.
13. **Mederski-Samorj, B. D., and B. E. Murray.** 1983. High level resistance to gentamicin in clinical isolates of enterococci. J. Infect. Dis. **147**:751–757.
14. **Miles, A. A., and S. S. Misva.** 1938. The estimation of the bactericidal power of the blood. J. Hyg. **38**:732–748.
15. **Murray, B. E., and B. Mederski-Samaroj.** 1983. Transferable beta-lactamase: a new mechanism for in vitro penicillin resistance in *Streptococcus faecalis*. J. Clin. Invest. **72**:1168–1171.
16. **National Committee for Clinical Laboratory Standards.** 1985. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.
17. **Sande, M. A., and W. M. Scheld.** 1980. Combination antibiotic therapy of bacterial endocarditis. Ann. Intern. Med. **92**: 390–395.
18. **Storch, G. A., and D. J. Krogstad.** 1981. Antibiotic-induced lysis of enterococci. J. Clin. Invest. **68**:639–645.
19. **Watanakunakorn, C., and C. Bakie.** 1973. Synergism of vancomycin-gentamicin and vancomycin-streptomycin against enterococci. Antimicrob. Agents Chemother. **4**:120–124.
20. **Wilkowske, C. J.** 1982. Enterococcal endocarditis. Mayo Clin. Proc. **57**:101–105.
21. **Wilson, W. R., and J. E. Gerçi.** 1983. Antibiotic treatment of infective endocarditis. Annu. Rev. Med. **34**:413–427.
22. **Wilson, W. R., C. J. Wilkowske, A. J. Wright, M. A. Sande, and J. E. Geraci.** 1984. Treatment of streptomycin-susceptible and streptomycin-resistant enterococcal endocarditis. Ann. Intern. Med. **100**:816–823.