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Bactericidal Activities of Peptide Antibiotics against Multidrug-Resistant *Enterococcus faecium*

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Multidrug-resistant *Enterococcus faecium* has emerged as a serious pathogen for which no effective therapy has been established. In this report, we describe the activities of two peptide antibiotics, ramoplanin and daptomycin, against 15 isolates of *E. faecium* resistant to vancomycin, ampicillin, and aminoglycosides using time-kill experiments. Both antibiotics were rapidly bactericidal when tested in broth; however, the addition of 50% serum resulted in significant regrowth. The combination of ampicillin with either ramoplanin or daptomycin largely prevented this regrowth. These peptide antibiotics showed good activity against these pathogens. While the development of daptomycin has been halted, ramoplanin may hold promise for the therapy of multidrug-resistant *E. faecium*, especially when combined with ampicillin.

Multidrug-resistant *Enterococcus faecium* has recently been declared the "nosocomial pathogen of the 1990s" (19). Increasing resistance to penicillin (9) and more recently to vancomycin (10, 14, 21) has been described elsewhere. Concomitant high-level resistance to aminoglycosides, vancomycin, and penicillin by *E. faecium* prevents synergy with aminoglycoside-cell wall agent combinations. Because there is no proven effective therapy, there is a need to find new antibiotic regimens for strains resistant to all available agents.

Novel peptide antibiotics with enhanced activity against many gram positive bacteria have been developed. Daptomycin is a cyclic lipopeptide which interferes with active transport of amino acids incorporated into the cell wall peptidoglycan (1). Because of poor initial results with daptomycin (7, 16), development of this drug has been halted. Ramoplanin is a lipoglycopeptide antibiotic which inhibits peptidoglycan synthesis by inhibiting the formation of lipid intermediate II (18). Cross-resistance between these drugs and older peptide antibiotics does not routinely occur (4, 11, 12). Therefore, these newer peptide antibiotics may retain activity against vancomycin-resistant strains. In this report, we describe the in vitro bactericidal activity of daptomycin and ramoplanin against 15 multidrug-resistant isolates of *E. faecium.*

From August 1991 through June 1992, six clinical isolates of *E. faecium* resistant to ampicillin, vancomycin, and gentamicin were collected at our hospital, and five additional isolates of *E. faecium* were obtained from patients at a neighboring community hospital in Brooklyn, N.Y. All but one were obtained from patients with bacteremia. In addition, four resistant isolates were generous gifts from the Nosocomial Pathogens Laboratory Branch, Centers for Disease Control, Atlanta, Ga. The organisms were identified as enterococci according to standard microbiological techniques and were identified to species level as *E. faecium* on the bases of carbohydrate fermentation, deamination of arginine, motility at 30°C, and pigment production according to the scheme described by Facklam and Collins (6).

Macrotube MICs were determined using an inoculum of approximately 5×10^5 CFU/ml in cation-supplemented Mueller-Hinton broth (50 mg of Ca^{2+} and 25 mg of Mg^{2+} per liter). The following antibiotics were tested: vancomycin and daptomycin (Eli Lilly Co., Indianapolis, Ind.), streptomycin (Pfizer Inc., Groton, Conn.), ampicillin (Roerig Division, Pfizer Inc., New York, N.Y.), ciprofloxacin (Miles Inc., West Haven, Conn.), gentamicin (Schering-Plough Corp., Bloomfield, N.J.), and ramoplanin and teicoplanin (Marion Merrell Dow, Cincinnati, Ohio). The effect of 10% pooled human serum on the MIC of ramoplanin was also determined. The presence of penicillinase was determined by nitrocefin disk testing (Becton Dickinson, Cockeysville, Md.). Kill-kinetic studies were performed using log-phase cultures adjusted to approximately 10^6 CFU/ml in supplemented Mueller-Hinton broth. The concentrations tested were 4× the MIC for daptomycin, a concentration previously shown to be maximally bactericidal for most enterococci (20); 1×, 2×, and 4× the MIC for ramoplanin; 20 μg/ml for vancomycin; 40 μg/ml for ampicillin; and 3 μg/ml for ciprofloxacin. The concentrations of the currently available antibiotics were chosen because they reflected medium to maximal clinically achievable blood concentrations. In addition, the effects of combining ramoplanin or daptomycin with vancomycin, ampicillin, and ciprofloxacin in the kill-kinetic studies were determined, and the effect of protein binding for selected antimicrobial agents was assessed by using broth containing 50% pooled human serum. Separate studies revealed that antibiotic carryover effects were present in undiluted samples of ramoplanin and daptomycin at the concentrations used in the kill-kinetic studies but were eliminated with a 10-fold dilution. Therefore, only 10-fold dilutions were used to determine the lowest number of detectable organisms. Bactericidal killing was defined as a killing of greater than 3 log by an antibiotic or combination at 24 h. Results are expressed as mean change $\log_{10}$ CFU/ml ± standard deviation. Student's *t* test for paired data was used for statistical analysis.

The MICs for the 15 isolates were ≥64 μg/ml for both ampicillin and vancomycin (Table 1). None were found to produce penicillinase. Seven of 15 isolates were resistant to teicoplanin (MIC, >8 μg/ml), and all 15 expressed high-level resistance to gentamicin and streptomycin (MIC, >2,000 μg/ml). The MICs of ramoplanin were the lowest, ranging from 0.5 to 1.0 μg/ml for all isolates (Table 1). In 10% serum, the MICs of ramoplanin generally increased fourfold; the MICs for 50 and 90% of the isolates were 2 and 4 μg/ml, respectively.

Time-kill studies revealed ramoplanin to be bactericidal in 11 of 15 isolates (Table 2). Suprainhibitory concentrations of ramoplanin (4× MIC) were only slightly superior to inhibitory

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TABLE 1. Susceptibility data for selected antimicrobial agents against 15 isolates of multidrug-resistant *E. faecium*

Antimicrobial agent	MIC (μg/ml)[a]		
	50%	90%	Range
Ampicillin	64	256	64–256
Vancomycin	512	1,024	64–1,024
Teicoplanin	8	1,024	1–1,024
Ciprofloxacin	4	>32	2–>32
Daptomycin	4	4	2–4
Ramoplanin	1	1	0.5–1
Ramoplanin with 10% serum	2	4	2–4

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

concentrations (1× MIC), with a change in mean $\log_{10}$ CFU/ml at 24 h of -3.3 ± 0.3 versus -3.0 ± 0.8, respectively. In comparison, daptomycin (at 4× MIC) produced a change in $\log_{10}$ CFU/ml of -3.3 ± 0.4 at 24 h and was bactericidal for 13 of 15 isolates. Ampicillin, vancomycin, and ciprofloxacin caused no reduction in colony counts when used alone.

Combinations of ampicillin, vancomycin, or ciprofloxacin with ramoplanin (latter concentration, 2× MIC) resulted in enhanced killing of the isolates (Table 2). The combination of ampicillin and ramoplanin produced significantly better killing than ramoplanin alone at 4 h (-3.5 ± 0.3 versus -2.9 ± 0.7 [$P < 0.01$]) and at 24 h (-3.7 ± 0.2 versus -3.1 ± 0.8 [$P = 0.01$]) and was bactericidal for all 15 isolates. The combination of vancomycin with ramoplanin was also bactericidal in all 15 isolates. The combination of ciprofloxacin with ramoplanin was only slightly better than ramoplanin alone.

When time-kill experiments with 50% serum were performed, there was a marked decrease in the killing activities of both ramoplanin and daptomycin. With concentrations of 4× MIC, substantial regrowth of organisms had occurred by 24 h (Table 2). Regrowth of at least 1 $\log_{10}$ CFU/ml following initial suppression or killing occurred in 13 of 15 isolates with ramoplanin and in 6 of 15 isolates with daptomycin. The combination of ampicillin (40 μg/ml) and ramoplanin produced greater killing than ramoplanin alone ($P < 0.001$), was bactericidal in 7 of 15 isolates, and permitted regrowth in only 1 of 15. The addition of ampicillin to daptomycin also resulted in greater killing in 50% serum than that with daptomycin alone ($P < 0.001$), was bactericidal in 11 of 15 isolates, and resulted in regrowth in 0 of 15. When ramoplanin was combined with penicillin (20 μg/ml) in serum, regrowth was again prevented (data not shown).

TABLE 2. Kill-kinetic studies of multidrug-resistant *E. faecium*

Antibiotic[a]	Mean change $\log_{10}$ CFU ± SD		No. of isolates for which antibiotic was bactericidal (n = 15)
	4 h	24 h	
GCNT	+2.2 ± 0.3	+2.6 ± 0.3	0
AMPI	+1.2 ± 0.5	+0.7 ± 1.1	0
CIP	−0.1 ± 1.5	+0.8 ± 1.6	0
VANC	+0.8 ± 1.0	+2.0 ± 0.7	0
DAPT4	−3.2 ± 0.5	−3.3 ± 0.4	13
RAMO1	−2.8 ± 0.7	−3.0 ± 0.8	9
RAMO2	−2.9 ± 0.7	−3.1 ± 0.8	10
RAMO4	−3.0 ± 0.6	−3.3 ± 0.6	11
RAMO2 + CIP	−3.4 ± 0.5	−3.4 ± 0.7	11
RAMO2 + VANC	−3.3 ± 0.7	−3.7 ± 0.2	15
RAMO2 + AMPI	−3.5 ± 0.3	−3.7 ± 0.2	15
GCNT[b]	+2.1 ± 0.3	+2.3 ± 0.4	0
AMPI[b]	+0.4 ± 0.7	+1.4 ± 0.7	0
RAMO4[b]	−0.8 ± 0.5	+0.8 ± 1.1	0
RAMO4 + AMPI[b]	−1.7 ± 0.8	−3.0 ± 0.9	7
DAPT4[b]	−2.1 ± 0.6	−1.5 ± 1.8	4
DAPT4 + AMPI[b]	−2.6 ± 0.6	−3.2 ± 0.8	11

[a] GCNT, growth control; AMPI, ampicillin (40 μg/ml); CIP, ciprofloxacin (3 μg/ml); VANC, vancomycin (20 μg/ml); RAMO1, -2 and -4, ramoplanin (1×, 2×, and 4× MIC, respectively); DAPT4, daptomycin (4× MIC).
[b] In the presence of 50% serum.

Enterococci have emerged as significant nosocomial pathogens. Proven antibiotic regimens have not been established for multidrug-resistant enterococci, and new and unconventional antimicrobial agents will almost certainly be necessary for effective therapy. The fact that 10 of our 11 clinical isolates originated from bacteremic patients underscores the need to urgently develop effective antimicrobial agents.

Daptomycin has demonstrated excellent in vitro activity against gram-positive bacteria, including enterococci (2, 5, 12, 15). However, the in vivo effectiveness of daptomycin has been disappointing (7, 16), presumably because of the high degree of protein binding of daptomycin (8, 13). These considerations may have discouraged further development of this drug. The present study found daptomycin to have similarly poor activity in vitro in the presence of serum. However, this investigation demonstrated that the combination of daptomycin with ampicillin provided effective killing of our highly resistant isolates, even in the presence of serum. Bingen et al. also found enhanced activity with the combination of daptomycin and ampicillin in broth against vancomycin-resistant *E. faecium* (3).

Ramoplanin is a new lipoglycopeptide antibiotic with activity against most gram-positive organisms (2, 17), including enterococci resistant to penicillin or vancomycin (11). Bactericidal activity against a small number of vancomycin-resistant enterococci has been demonstrated elsewhere (4, 11). Although we found similar effectiveness in broth, the addition of serum eliminated killing activity even at suprainhibitory concentrations (4× MIC). However, the addition of ampicillin to ramoplanin was effective, even in the presence of serum. Given its in vitro activity against resistant enterococci, further evaluation of ramoplanin for systemic administration appears warranted.

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