Efficacy of Vancomycin and Teicoplanin Alone and in Combination with Streptomycin in Experimental, Low-Level Vancomycin-Resistant, VanB-Type Enterococcus faecalis Endocarditis

DAVID P. NICOLAU,1*, MARKOS N. MARANGOS,1 CHARLES H. NIGHTINGALE,1 KALPANA B. PATEL,1 BRIAN W. COOPER,1 RICHARD QUINTILIANI, JR.,2 PATRICE COURVALIN,2 and RICHARD QUINTILIANI1

Hartford Hospital, Hartford, Connecticut,1 and Institut Pasteur, Paris, France2

Received 25 July 1995/Returned for modification 29 August 1995/Accepted 20 October 1995

The efficacy of vancomycin (VM) and teicoplanin (TE), alone and in combination with streptomycin (SM), against enterococci that express low-level VanB-type VM resistance was investigated in experimental endocarditis using isogenic strains of Enterococcus faecalis susceptible to glycopeptides and aminoglycosides or inducibly resistant to low levels of VM (MIC = 16 μg/ml). VM was significantly less active against the resistant strain than against the susceptible strain, establishing that low-level VanB-type VM resistance can influence therapeutic efficacy. By contrast, TE had equally good activity against both strains. VM or TE combined with SM was synergistic and bactericidal against the resistant strain in vitro. While both combinations were efficient in reducing bacterial density in vivo, TE plus SM was significantly superior to VM plus SM if valve sterilization was considered. These data suggest that despite the presence of low-level VanB-type resistance, combination therapy with a glycopeptide and SM (and presumably other aminoglycosides to which there is not high-level resistance) will nevertheless provide effective bactericidal activity.

Enterococcus faecalis and Enterococcus faecium are important causes of intra-abdominal, pelvic, urinary tract, and cardiac infections as well as the second most common bacteria responsible for nosocomial infections (30). Although there has never been a controlled trial of combination therapy versus monotherapy for enterococcal endocarditis, it is generally agreed that serious enterococcal infections, such as endocarditis and meningitis, require administration of a synergistic combination of a cell wall-active agent, usually a penicillin or vancomycin (VM), with an aminoglycoside to achieve maximum rates of cure. However, the synergy and bactericidal activity of these combinations are lost if the strain is highly resistant to one of the antimicrobial agents (3, 15, 16, 20, 31). Therapy of enterococcal infections has therefore become complicated, since many of the strains have acquired genes or techniques perform poorly in the detection of low-level VM resistance. VanB-type strains may be more prevalent than previously believed (27, 28, 32, 33, 36). For these reasons, infections due to such strains may well be, albeit unknowingly, treated inadequately. However, the clinical relevance of low-level VanB-type resistance has not yet been determined. Moreover, although it is clear that high-level resistance to VM abolishes synergy between this antibiotic and an aminoglycoside (15), loss of antimicrobial synergy against VanB-type strains expressing inducible, low-level resistance has also not been examined. In a previous study intended to address these questions (9), the E. faecium strain studied was reidentified as Enterococcus gallinarum, a species intrinsically resistant to VM (VanC phenotype) (14a).

Of major clinical importance, certain enterococcal strains harboring vanB may be classified as susceptible by MIC determination (25). In addition, since antibiotic susceptibility techniques perform poorly in the detection of low-level VM resistance, VanB-type strains may be more prevalent than previously believed (27, 28, 32, 33, 36). For these reasons, infections due to such strains may well be, albeit unknowingly, treated inadequately. However, the clinical relevance of low-level VanB-type resistance has not yet been determined. Moreover, although it is clear that high-level resistance to VM abolishes synergy between this antibiotic and an aminoglycoside (15), loss of antimicrobial synergy against VanB-type strains expressing inducible, low-level resistance has also not been examined. In a previous study intended to address these questions (9), the E. faecium strain studied was reidentified as Enterococcus gallinarum, a species intrinsically resistant to VM (VanC phenotype) (14a).

The purpose of this study was to determine the efficacy of VM and TE, alone or in combination with streptomycin (SM), against low-level VM-resistant, VanB-type E. faecalis in a rabbit model of endocarditis. We chose to evaluate SM in combination with VM or TE since among 20 geographically diverse VanB-type strains for which the MICs of VM were ≤ 32 μg/ml, 50% were also resistant to high levels of SM, 65% were resistant to gentamicin, and 85% were resistant to kanamycin.

(This research was presented in part at the 34th Interscience Conference on Antimicrobial Agents and Chemotherapy, Orlando, Fla., October 1994 [23a].)
Bacterial strains. E. faecalis HH260 is a VanB-type clinical isolate from Hartford Hospital, harboring vanB-related sequences (25). The resistant strain of each drug was determined by the microdilution technique with Mueller-Hinton broth (Difco). The susceptible control strain E. faecalis BM4296 was rifampin- and fusidic acid-resistant mutant of E. faecalis 309 that was obtained by a selection on brain heart infusion agar (Difco, Detroit, Mich.) supplemented with rifampin (20 μg/ml) or fusidic acid (10 μg/ml). The VM-resistant transconjugant BM4297 was obtained by mating on filters (25) E. faecalis HH260 with E. faecalis BM4296 with selective agar containing rifampin (20 μg/ml), fusidic acid (10 μg/ml), and VM (8 μg/ml). Discrete susceptibility testing indicated that BM4297 had acquired only VM resistance. The MIC of VM for E. faecalis BM4296 was 16 μg/ml, identical to that of the donor.

The presence of nucleotide sequences related to vanB in these strains was studied by DNA-DNA hybridization (25). A probe specific for vanB (8) hybridized with a 3.3-kb HindIII-KpnI fragment of total DNA from HH260 and BM4297 but not with that of BM4296 (data not shown). No loss of VM resistance by BM4297 was observed after seven consecutive overnight subcultures of BM4297 in 100 ml of antibiotic-free Mueller-Hinton broth (Difco).

In vitro susceptibility to antibiotics. MICs of VM (Eli Lilly, Indianapolis, Ind.), TE (Marion Merrell Dow Inc., Cincinnati, Ohio), and SM (Pfizer Laboratory, New York, N.Y.) were determined by microdilution technique with an inoculum of 5 × 10^6 CFU/ml in Mueller-Hinton broth (29).

Study of combined antibacterial activity. Time-kill curve methods were used to evaluate the bactericidal activities of VM and TE, alone or in combination with VM. The in vitro antibacterial concentrations were selected to cover the typical peak and trough levels: VM, 50 and 10 μg/ml; TE, 50 and 20 μg/ml; and SM, 30 and 5 μg/ml. Experiments were performed with Mueller-Hinton broth with an inoculum of 5 × 10^5 CFU/ml and samples (10 μl) taken after 0, 2, 4, 8, 12, and 24 h for colony counts. Synergism was defined as a decrease of at least 2 log_{10} CFU in CFU per milliliter at 24 h between the antimicrobial combination and its more active component, with at least one of the drugs not altering the growth curve. Bactericidal activity was defined as a decrease of at least 3 log_{10} CFU/ml in bacterial cell density after 24 h of incubation. The limit of sensitivity was 100 CFU/ml. Drug carryover was avoided by serial dilution and spreading on agar plates. Experimental endocarditis. Experiments were performed with female New Zealand White rabbits weighing between 2.5 and 3 kg. Aortic endocarditis was induced by inserting a polyethylene catheter into the right internal carotid artery and advancing it over the aortic valve, as described previously (23). Ninety-six hours after catheter insertion, the animals were inoculated by the marginal ear vein with 10^7 CFU of E. faecalis BM4296 or BM4297. Twenty-four hours after inoculation, the rabbits received one of the following 3-day treatment regimens: VM at 50 mg/kg of body weight given intravenously every 8 h, TE at 20 mg/kg given intravenously every 12 h, SM at 3.5 mg/kg given intramuscularly every 8 h, or a combination of VM plus TE at the same dosages. Control animals were left untreated. Fifty-one of 72 rabbits designated to receive E. faecalis BM4296 and randomized to a non-SM-containing treatment regimen (control, n = 20; VM, n = 16; and TE, n = 15) completed the regimen, while 54 of 74 assigned to receive E. faecalis BM4297 (control, n = 18; VM, n = 11; and TE, n = 15) completed the regimen. Twenty-eight of 34 animals designated to receive E. faecalis BM4296 and an SM-containing regimen, 28 completed the treatment regimen (SM, n = 9; SM plus VM, n = 11; and SM plus TE, n = 8). Failure to complete the 3-day regimen was due to animal death before inoculation of the test strains or improper placement of the catheter determined at autopsy.

The antimicrobial agent doses and dosing intervals were chosen to simulate human pharmacokinetics (peak and trough levels in serum, respectively: VM, 9 and <1 μg/ml; TE, 50 and 20 μg/ml; and SM, 50 and 10 μg/ml) and were based on studies previously completed in our laboratory and in other laboratories (9, 14, 21, 22). Since we had not previously dosed TE in the rabbit model, trough concentrations in serum were determined 12 h after the dose by bioassay (6). This assay was linear over a range of 2 to 50 μg/ml, with interday and intraday coefficients of variation for both quality control samples (3 and 45 μg/ml) of ±12 and ±4%, respectively. The mean TE trough concentration ± standard deviation was 2.3 ± 1.5 μg/ml, value comparable to that obtained by others using similar dosing regimens (9).

Once dosing had been completed, the animals were sacrificed at either 8 or 12 h after the last dose, depending on the treatment interval. Sacrifice was undertaken by injection of pentothal sodium followed by potassium chloride. After sacrifice, the hearts were removed aseptically and the chambers on the left side were examined. Aortic vegetations were excised, pooled, washed in sterile saline, and blotted dry on filter paper. CFU were determined by homogenization (1:10 v/v) of sterile 0.9% NaCl. Serial dilution and plating techniques were used to determine the number of CFU present after incubation at 37°C for 48 h. Colony counts were expressed as log_{10} CFU per gram of vegetation. Vegetations were considered sterile (limit of sensitivity, 100 CFU per vegetation) if there was no growth after 48 h of incubation. Culture-negative specimens were considered to contain 100 CFU for comparison with other

---

RESULTS

In vitro susceptibility to antibiotics. Acquisition of the vanB gene cluster by E. faecalis BM4296, resulting in strain BM4297, led to an eightfold increase in the MIC of VM (2 to 16 μg/ml), whereas the MIC of TE (0.25 μg/ml) remained unchanged. Both strains were intrinsically resistant to low levels of SM (MIC = 256 μg/ml).

Study of combined antimicrobial activity. Time-kill curves were determined with VM or TE, tested alone or in combination with SM against E. faecalis BM4296 (Fig. 1) and E. faecalis BM4297 (Fig. 2). As expected, SM alone at a low (5 μg/ml) or a high (30 μg/ml) concentration showed no antimicrobial activity against either strain. VM was bacteriostatic against E. faecalis BM4296 regardless of the antimicrobial concentration. By contrast, whereas VM at a concentration of 50 μg/ml was bacteriostatic against VM-resistant BM4297, an increase in growth of 2.0 log_{10} CFU/ml was observed after 24 h at 10 μg/ml. Both low (20 μg/ml) and high (50 μg/ml) concentrations of TE were bacteriostatic against the susceptible strain and the resistant strain.

VM or TE combined with SM resulted in bactericidal synergism against E. faecalis BM4296 for all combinations tested. TE plus SM achieved more-rapid killing than VM plus SM, and except for the combination including low concentrations of TE (20 μg/ml) and SM (5 μg/ml), no growth was detected after 12 h of incubation. When tested against E. faecalis BM4297, VM at 50 μg/ml combined with either low- or high-concentration SM achieved bacteriostatic synergy. Although VM at 10 μg/ml combined with a low or high concentration of SM initially appeared synergistic, significant bacterial growth occurred by 24 h of incubation. By contrast, all combinations of TE and SM, irrespective of the concentrations tested, were bactericidal against E. faecalis BM4297, with no detectable growth after 12 h. The susceptibility to VM and TE of strains that displayed significant regrowth was not changed, indicating that this phenomenon was not due to the emergence of constitutively resistant mutants.

Experimental aortic endocarditis (i) VM, TE, or no treatment against E. faecalis BM4296 and BM4297 (Table 1). There was no significant difference in CFU per gram of vegetation between untreated rabbits inoculated with E. faecalis BM4296 and BM4297. This result was expected, given the similar genetic backgrounds of these strains. Among rabbits inoculated with BM4296, VM and TE achieved similar bacterial reductions of 3.4 and 2.8 log_{10} CFU/g of vegetation, respectively, compared with the level in untreated controls. VM was significantly more effective against BM4296 than against BM4297, producing a reduction in bacterial density that was 2.2 log_{10} CFU/g of sterile 0.9% NaCl, after 24 h of incubation in the former strain. By contrast, TE was equally active against E. faecalis BM4296 and BM4297, achieving reductions in bacterial density of 2.8 and 2.9 log_{10} CFU/g of vegetation, respectively, compared with the level in untreated controls. Thus, the efficacy of treatment with VM,
but not that with TE, was diminished by low-level VanB-type VM resistance.

(ii) VM and TE, alone and in combination with SM against E. faecalis BM4297 (Table 1). SM alone had no activity against VM-resistant BM4297, with bacterial titers indistinguishable from those of untreated controls. Compared with SM alone, VM and TE each combined with SM produced similarly impressive bacterial reductions of 3.8 and 4.2 log₁₀ CFU/g of vegetation, respectively. However, when the results were expressed as percentages of animals with sterile vegetations, 75% treated with TE plus SM had sterile vegetations, whereas only 9% treated with VM plus SM did. Because bactericidal synergy, which has been demonstrated to be clinically efficacious, was not abolished by the presence of vanB in BM4297, and since the activity of combination therapy against susceptible enterococci, including BM4296 (10), is well established, similar experiments were not repeated with BM4296.

DISCUSSION

In a rabbit model of aortic endocarditis, VM alone reduced the bacterial density of cardiac vegetations induced by a VanB-
type *E. faecalis* strain expressing inducible, low-level VM resistance significantly less than that of its susceptible counterpart. This result indicates that in circumstances in which VM may be potentially administered alone, such as for infections due to enterococci with high-level aminoglycoside resistance, expression of low-level VanB-type VM resistance could result in increased therapeutic failure. This finding is significant, since these strains are difficult for the clinical microbiology laboratory to detect.

TE alone was more effective than VM alone against the resistant strain, achieving a reduction of nearly 3 log₁₀ CFU/g of vegetation compared with the level in untreated controls. The good in vivo activity of TE suggests that this antibiotic may be useful for the treatment of infections caused by low-level VM-resistant VanB strains, particularly those resistant to high levels of aminoglycosides, and is consistent with the demonstrated potential of TE alone for curing patients with VM-susceptible *E. faecalis* endocarditis (24). Various investigators have suggested that high-dose TE is most likely to provide efficacy in vivo (9, 17, 38). It should be cautioned, however, that spontaneous mutants resistant to TE have been obtained in vitro. In addition, development of TE resistance has been...
reported for a patient infected with a VanB-type strain of *E. faecalis* who was treated with VM (12). TE is a glycopeptide antibiotic with a structure and spectrum of activity similar to those of VM, which has been available in Europe for several years. Although this drug is not approved for clinical use in the United States, approval for compassionate use can be requested. For VanB-type isolates that are resistant to high levels of aminoglycosides and that prove susceptible or moderately susceptible to penicillins, however, a prolonged course of parenteral ampicillin may also be a reasonable initial approach (11,13,34,35).

High-level resistance to VM abolishes bactericidal synergy between this class of antibiotic and an aminoglycoside. By contrast, our results indicate that bactericidal synergy can be obtained with VM combined with SM against a VanB-type *E. faecalis* strain expressing low-level VM resistance and intrinsically resistant to low levels of SM. In agreement with these findings, enhanced bactericidal activity was also observed in the same model between VM and gentamicin against an isolate of *E. gallinarum* intrinsically resistant to low levels of VM (VanC phenotype) and gentamicin (9). From a clinical perspective these results are fortunate, since despite the possibility of undetected VanB-type VM resistance, our data suggest that combination therapy with VM and SM (and presumably other aminoglycosides to which there is no high-level resistance) will nevertheless provide effective bactericidal therapy. Therapy with a combination of VM and an aminoglycoside may be possible for the majority of low-level VM-resistant VanB-type strains, since 70% of our isolates were susceptible to at least one clinically useful aminoglycoside. It should be emphasized, however, that for strains without high-level resistance to aminoglycosides which are susceptible or moderately susceptible to penicillin, the clinically well-established synergistic combination of a penicillin with an aminoglycoside should be used.

For the VM-plus-SM combination, the VM concentration appeared to be an important factor for in vitro efficacy. Antibacterial combinations that included a low concentration of VM which mimicked trough levels in serum allowed regrowth of the resistant strain. These data suggest that elevated trough VM levels may be therapeutically beneficial in the treatment of infections caused by these strains. The MICs of VM and TE for strains that exhibited significant growth in the presence of VM plus SM were unchanged, indicating that the poor response was not due to the emergence of constitutively resistant mutants. Induction of VM resistance, which causes cross-resistance to TE, may have been responsible for this phenomenon despite the fact that the strains were susceptible to TE, since deinduction occurs rapidly. SM combined with a high concentration of VM may have prevented induction of resistance.

The combination of TE plus SM achieved more-rapid killing than VM plus SM, and in terms of aortic valve sterilization, TE plus SM was significantly superior to VM plus SM. The potential for induction of resistance is also precluded since TE, unlike VM, does not induce expression of the vanB gene cluster. The potential for emergence of constitutively resistant mutants after exposure to glycopeptides, however, stresses the importance of repeat susceptibility testing of strains isolated from patients failing to respond to therapy. Whether the emergence of such mutants is diminished or prevented by effective combination therapy has not yet been studied.

**ACKNOWLEDGMENTS**

We thank members of the animal research facility at Hartford Hospital, M. Glauser for the gift of *E. faecalis* 309, E. H. W. Bohme and Marion Merrell Dow Inc. for kindly providing TE, Pfizer Laboratories for kindly providing SM, and M. Arthur and A. Smith for critical review of the manuscript.

This work was supported by grant 1017-80-065 from Hartford Hospital.

**REFERENCES**

14a. Leclercq, R. Personal communication.