Synergistic Activity between Vancomycin or Teicoplanin and Gentamicin or Tobramycin against Pathogenic Diphtheroids

PETER G. SPITZER,†* GEORGE M. ELIPOULOS, ADOLF W. KARCHMER, AND ROBERT C. MOELLERING, JR.

Department of Medicine, New England Deaconess Hospital, and Harvard Medical School, Boston, Massachusetts 02215

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The in vitro activities of vancomycin and teicoplanin alone and in combination with gentamicin or tobramycin were studied by time-kill techniques with 11 strains of pathogenic diphtheroids (Corynebacterium group JK). The activities of vancomycin and teicoplanin were similar (MIC for 90% of strains tested [MIC90], 1 μg/ml), as were those of gentamicin and tobramycin (the MIC90 was 1 μg/ml for five aminoglycoside-susceptible strains, and the MIC90 was >1.024 μg/ml for six aminoglycoside-resistant strains). No consistent synergistic killing could be demonstrated by the combination of glycopeptide and aminoglycoside antibiotics at arbitrarily chosen concentrations within the range of clinically achievable levels. However, by careful adjustment of both vancomycin and gentamicin concentrations within a narrow range below the MIC of each antibiotic, synergistic killing could be seen with an aminoglycoside-susceptible strain but not with an aminoglycoside-resistant strain. Synergism between glycopeptide and aminoglycoside antibiotics occurs with some diphtheroid organisms, but it may not be clinically relevant.

Diphtheroids, primarily Corynebacterium spp. other than Corynebacterium diphtheriae, are increasingly recognized as human pathogens (2, 3). As a group, pathogenic diphtheroids vary in antibiotic susceptibility patterns (3), and some strains, often designated Corynebacterium group JK, are resistant to most antibiotics tested except vancomycin (5, 8). Murray et al. (3) reported synergistic killing of several strains of diphtheroids by combinations of penicillin with gentamicin; however, the interaction between glycopeptide antibiotics and aminoglycosides has not been explored. The purpose of this study was to evaluate the activity of two glycopeptide antibiotics, vancomycin and teicoplanin, against pathogenic diphtheroids and to investigate the potential for bactericidal synergism between these compounds and gentamicin or tobramycin.

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MATERIALS AND METHODS

Organisms. Eleven strains of diphtheroids were studied. Four strains identified as Corynebacterium group JK were obtained from the Centers for Disease Control, Atlanta, Ga. Seven other strains from patients with prosthetic-valve endocarditis were identified previously as belonging to the JK group by Centers for Disease Control criteria (3). All 11 isolates had been stored at −70°C and grew aerobically. After being thawed, they were maintained on brucella agar plates with 5% horse blood (GIBCO Laboratories, Madison, Wis.).

Antimicrobial agents. Standard antimicrobial reference powders were obtained from the following sources: teicoplanin, Merrell Dow Pharmaceuticals, Inc., Cincinnati, Ohio; vancomycin hydrochloride, Eli Lilly & Co., Indianapolis, Ind.; gentamicin sulfate, Schering Pharmaceutical Corp., Manati, P.R.; and tobramycin sulfate, Distac Products Co., Carolina, P.R.

Susceptibility studies. MICs of teicoplanin, vancomycin, gentamicin, and tobramycin were determined by an agar dilution method (9) with brain heart infusion (BHI) agar (Difco Laboratories, Detroit, Mich.). Inocula were prepared from overnight cultures of test organisms in BHI broth (Difco) with 5% rabbit serum (Whittaker M.A. Bioproducts, Walkersville, Md.). Bacterial suspensions were applied with a 32-prong inoculator to yield inocula of ca. 10^6 CFU. Plates were examined after 24 and 48 h of incubation at 35°C in room air. The MIC was defined as the lowest concentration of drug which inhibited visible growth after 48 h of incubation. The endpoint of 48 h was chosen because of previously documented slow growth of some of these organisms (3).

Synergism studies. The bactericidal activities of vancomycin and teicoplanin alone and in combination with gentamicin or tobramycin against the 11 diphtheroid strains were determined by time-kill techniques. These studies were carried out in 250-ml flasks to which 19 ml of BHI broth with 5% rabbit serum, appropriate antibiotics (in ≤0.06 ml of distilled water), and 1 ml of an overnight culture of a diphtheroid in BHI broth with 5% rabbit serum were added. The final mixture contained approximately 10^6 CFU/ml. The flasks were loosely plugged and incubated without agitation at 35°C in room air. Samples (0.5 ml) were withdrawn for colony counts after 0, 24, and 48 h. The lower limit of detection of viable cells by this method was 20 CFU/ml (i.e., 1.3 log10 CFU/ml).

Synergism was defined as a 100-fold or greater enhancement of killing after 24 or 48 h of incubation by an antibiotic combination compared with the more effective of the antibiotics used alone.

Enzyme studies. The six aminoglycoside-resistant isolates...
were tested for the presence of aminoglycoside-modifying enzymes by methods previously described (3).

RESULTS

Agar dilution studies. Vancomycin (MIC for 90% of strains tested, 1.0 µg/ml) and teicoplanin (MIC for 90% of strains tested, 1.0 µg/ml) inhibited growth of all strains at concentrations between 0.5 and 2 µg/ml. Within the range of concentrations studied, MICs of gentamicin equaled those of tobramycin for all strains. Six strains were aminoglycoside resistant (MIC, >1.024 µg/ml), while five strains were aminoglycoside susceptible (MIC, 0.125 to 1 µg/ml).

Synergism studies. For each strain tested, the following antibiotic concentrations were used: vancomycin and teicoplanin, 1 µg/ml (chosen because this concentration inhibited growth of 90% of the strains); gentamicin and tobramycin, 5 µg/ml for aminoglycoside-resistant strains and ca. one-eighth of the MIC for aminoglycoside-susceptible strains. This fraction of the MIC for aminoglycoside-susceptible strains was chosen because higher concentrations of aminoglycosides alone (i.e., >one-eighth of the MIC) often caused some inhibition of growth which prevented reliable determinations of synergism. We excluded drug carry-over as a potential cause of inaccuracies in preliminary experiments with one strain each of an aminoglycoside-susceptible and an aminoglycoside-resistant diphtheroid. Organisms were diluted to a low inoculum (ca. 10^8 CFU/ml) and added to physiologic saline (the dilution medium) which contained a glycopeptide either alone or combined with an aminoglycoside at the highest concentrations employed in synergy experiments. Duplicate samples (0.025 ml) were spotted onto antibiotic-free plates, and colony counts were compared with those obtained with control specimens processed in the same manner except without exposure to antibiotics. In no case was the colony count obtained after antibiotic exposure lower than that with control samples by more than 0.2 log_{10} CFU/ml.

Combinations of glycopeptide and aminoglycoside antibiotics failed to show consistent synergistic killing of either aminoglycoside-susceptible or -resistant strains at the concentrations of antibiotics arbitrarily chosen. These results are summarized in Table 1.

Additional time-kill studies. (i) Synergism demonstrated. To determine whether failure to detect synergism against aminoglycoside-susceptible strains was coincidentally due to the arbitrary choice of antibiotic concentrations employed or was truly due to a lack of drug interaction, one isolate demonstrating aminoglycoside susceptibility was examined in detail. By careful adjustment of both glycopeptide and aminoglycoside antibiotics within a narrow range of concentrations below the MIC of each, synergism could be demonstrated (Fig. 1). However, manipulations of drug concentrations failed to detect synergistic interactions against an aminoglycoside-resistant strain.

(ii) Glycopeptide antibiotics more effective against aminoglycoside-susceptible strains than against aminoglycoside-resistant strains. Both glycopeptide antibiotics demonstrated greater degrees of killing of aminoglycoside-susceptible organisms than of aminoglycoside-resistant organisms (Table 1). This phenomenon was further investigated by performing detailed time-kill studies of the effects of various concentrations of vancomycin on eight diphtheroid strains (four aminoglycoside-resistant strains and four aminoglycoside-susceptible strains), each strain susceptible to the same MIC of vancomycin. An aminoglycoside-susceptible strain was paired with an arbitrarily chosen aminoglycoside-resistant strain for each experiment carried out on a single day. Vancomycin concentrations tested were always the same for the two strains and included concentrations below the MIC (0.5 µg/ml), at the MIC (1 µg/ml), and above the MIC (2 µg/ml) for the four sets of paired organisms. For vancomycin concentrations at or above the MIC for an organism, bactericidal activity determined at 24 h was greater for aminoglycoside-susceptible than for aminoglycoside-resistant strains. This was also true at the 48-h sample time for three of the four pairs of strains. For the fourth pair, colony counts at 48 h were too near the lower limits of detection to allow reliable assessment. The magnitude of increased killing seen with aminoglycoside-susceptible organisms relative to that seen with aminoglycoside-resistant organisms ranged from 1...
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FIG. 1. Effect of vancomycin and gentamicin alone and in combination against strain 17. The MIC of vancomycin (VM) was 1.0 μg/ml; the MIC of gentamicin (GM) was 0.125 μg/ml. Drug concentrations shown are in micrograms per milliliter. The lower limit of detection of viable bacteria was 1.3 log_{10} CFU/ml.

to 4 log_{10} CFU/ml. At vancomycin concentrations below the MIC for an organism, there was no difference in growth between aminoglycoside-susceptible and -resistant strains. The time-kill curves for one set of paired organisms are shown in Fig. 2.

Enzyme studies. There were no detectable aminoglycoside-modifying enzymes in any of the six aminoglycoside-resistant isolates when kanamycin and gentamicin were used as substrates in tests for acetyltransferase and phosphotransferase activities and when streptomycin and gentamicin were used as substrates in tests for adényllyltransferase activity.

DISCUSSION

All 11 strains of pathogenic group JK diphtheroids were susceptible to vancomycin and teicoplanin. Activities of the two agents were comparable against these isolates. This finding is consistent with previously reported results (1, 4). In addition, we found the activity of tobramycin to be virtually identical to that of gentamicin.

The finding that some diphtheroid organisms are resistant to high concentrations of aminoglycoside antibiotics is in agreement with previously reported results (6). The mechanism of resistance to aminoglycosides in Corynebacterium group JK is unknown. While a plasmid apparently mediating kanamycin resistance in a non-group JK coryneform bacterium from human skin has been described elsewhere (7), plasmid-mediated resistance to gentamicin or tobramycin has not been identified in group JK diphtheroids (8). In the present study, aminoglycoside-modifying enzymes could not be detected. While aminoglycoside resistance based on alterations of ribosomal target sites is possible, resistance based on diminished penetration of aminoglycosides into the organisms seems more likely, given the patterns of multiple antibiotic resistance observed in many of these strains.

While few data exist on the in vivo effectiveness of synergistic combinations of antibiotics for the therapy of diphtheroid infections, many patients infected with these organisms are quite ill, are often immunosuppressed, and may have indwelling prosthetic material. In these cases, the use of a bactericidal regimen would seem attractive. Many strains of diphtheroids are resistant to all available antibiotics except vancomycin (5, 8). However, vancomycin may not always be bactericidal in vitro, even at concentrations 16 times that of the MIC for a particular organism (3). For this reason, antibiotic combinations that produce bactericidal synergism may be important.

In this study, with the initial concentrations of antibiotics used (arbitrarily selected as being within a relevant clinical range), synergistic killing by combinations of glycopeptide and aminoglycoside antibiotics could not be consistently demonstrated. However, synergistic killing could be detected against an aminoglycoside-susceptible strain by careful adjustment of both glycopeptide and aminoglycoside antibiotics within a narrow range of concentrations below the MIC of each antibiotic. No evidence of true synergistic killing was ever detected against aminoglycoside-resistant strains. These data parallel the results of Murray et al. (3), who found that penicillin-gentamicin combinations produced bactericidal synergism only against gentamicin-susceptible diphtheroids.

These data indicate that determination of gentamicin or tobramycin susceptibility of clinically significant isolates of group JK corynebacteria would provide useful information to clinicians. For aminoglycoside-susceptible strains, syner-

FIG. 2. Bactericidal activity of vancomycin (VM) against an aminoglycoside-susceptible diphtheroid (●) and an aminoglycoside-resistant strain (○). The MIC of vancomycin for both isolates was 1.0 μg/ml. Drug concentrations shown are in micrograms per milliliter. The lower limit of detection of viable bacteria was 1.3 log_{10} CFU/ml.
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LITERATURE CITED