Vancomycin-Gentamicin Synergism Revisited: Effect of Gentamicin Susceptibility of Methicillin-Resistant Staphylococcus aureus

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Vancomycin monotherapy of deep-seated staphylococcal infection may be associated with poor bacteriologic response. We evaluated 24 unique patient isolates of methicillin-resistant Staphylococcus aureus (MRSA) for vancomycin-gentamicin synergism by determining time-kill curves for vancomycin at 10 μg/ml and gentamicin at 1 μg/ml. Nine MRSA strains showed high-level gentamicin resistance (HLGR) (MIC, >500 μg/ml), and 15 did not. Vancomycin-gentamicin demonstrated synergism against none of the HLGR strains. For the non-HLGR strains, gentamicin agar dilution MICs ranged from 0.5 to >128 μg/ml. Vancomycin-gentamicin demonstrated synergism against six of these strains and indifference against nine of them. There was no relationship between the agar dilution MIC of gentamicin and the occurrence of synergism against non-HLGR strains. We conclude that a gentamicin MIC of >500 μg/ml predicts a lack of vancomycin-gentamicin synergism for strains of MRSA. For non-HLGR strains, synergism is not predictable from the gentamicin MIC.

Therapeutic options for deep-seated infection due to methicillin-resistant Staphylococcus aureus (MRSA) are limited. Although intravenously administered vancomycin is the drug of choice for the treatment of MRSA infections, treatment failures with vancomycin monotherapy of staphylococcal endocarditis have been reported (4, 7). In treating complicated MRSA infections, many clinicians combine vancomycin with another agent, such as gentamicin, in the expectation of a more rapid bacteriologic response based on a synergistic interaction between the two antibiotics. Overall, there are few data comparing vancomycin monotherapy with vancomycin in combination with aminoglycosides for the treatment of severe MRSA infections.

The relationship between gentamicin susceptibility and vancomycin-gentamicin synergism is not defined for staphylococci. In enterococci, high-level gentamicin resistance (HLGR) (MIC, >500 μg/ml by agar dilution) predicts the lack of a synergistic interaction between a cell wall active agent and gentamicin. Enterococcal strains for which the gentamicin MIC is <500 μg/ml routinely show such synergism. There are no comparable data for MRSA, despite the fact that the combination of vancomycin and gentamicin is frequently used in the treatment of life-threatening MRSA infections. We performed time-kill synergism studies with strains of MRSA with various levels of gentamicin resistance to assess the relationship between gentamicin susceptibility and vancomycin-gentamicin synergism in staphylococci. We specifically sought to determine whether HLGR, as defined for enterococci, predicts a lack of vancomycin-gentamicin synergism in staphylococci.

MATERIALS AND METHODS

Bacteria. The 24 MRSA strains used in this study were clinical isolates obtained from unique patients at the Veterans Affairs Medical Center, Pittsburgh, Pa., from 1993 to 1994.

Method. Twenty-four MRSA isolates were screened for HLGR by using the agar screening method with brain heart infusion agar (Remel) containing 500 μg of gentamicin per ml as recommended by the National Committee for Clinical Laboratory Standards guidelines (6). Gentamicin MICs were determined by agar dilution in accordance with National Committee for Clinical Laboratory Standards guidelines and tables.

Time-kill method. A standard time-kill method (1) was used to study the interaction between vancomycin and gentamicin for HLGR and non-HLGR strains of MRSA. The drug concentrations that were chosen represent achievable concentrations in serum: 10 μg/ml for vancomycin and 1 μg/ml for gentamicin. A broth culture with no antibiotic was set up as a control. The inoculum was 10⁶ to 10⁹ CFU/ml. Undiluted and serially diluted samples were plated for colony counting. The procedure was duplicated for determination of reproducibility. The lowest detectable number of organisms was 10 CFU/ml. A drug carryover effect was excluded by showing that there was a less than 5% difference in colony counts when 10 μl of a 10⁻⁶ to 10⁻⁹ CFU/ml inoculum was plated onto antibiotic-free plates with or without vancomycin (10 μg/ml) and gentamicin (1 μg/ml) in the aliquots. Colony counts were made at 0, 4, and 24 h after incubation at 35°C. Synergism was defined as a decrease in colony counts of at least 100-fold at 24 h with the combination compared with that of the most active single drug.

Antagonism was defined as an increase in colony counts of at least 100-fold at 24 h with the combination compared with that of the most active single drug. Indifference was defined as an increase or a decrease in colony counts of less than 100-fold at 24 h with the combination compared with that of the most active single drug.

RESULTS

Nine strains were resistant to gentamicin at 500 μg/ml, and 15 strains were susceptible at this concentration. None of the nine HLGR MRSA strains demonstrated vancomycin-gentamicin synergism. Of the 15 remaining strains not showing HLGR 6 were synergistically affected (Fig. 1). For all of the other strains, the drugs showed indifference (Fig. 2).

There was no consistent relationship involving gentamicin susceptibility as determined by agar diffusion for non-HLGR strains of MRSA. MICs for the strains for which the drugs demonstrated synergism ranged from 16 to >128 μg/ml. MICs for the strains for which the drugs showed indifference ranged from 0.5 to >128 μg/ml. Two of the latter were susceptible to gentamicin by National Committee for Clinical Laboratory Standards criteria, with MICs of 0.5 and 2 μg/ml, respectively.

DISCUSSION

Vancomycin is the mainstay of therapy of MRSA infection. However, vancomycin treatment of deep-seated staphylococcal infections such as endocarditis has been associated with slow
demonstrated. Vancomycin is less rapidly bactericidal in vitro against methicillin-susceptible strains, indifference was demonstrated. Nakorn and Tisone (8) concluded that “the combination of vancomycin-gentamicin or vancomycin-tobramycin were shown to be synergistic against a majority of methicillin-susceptible and methicillin-resistant strains of Staphylococcus aureus.” A close examination of the data presented in that study shows that most of the MRSA strains studied were not, in fact, synergistically affected by the antibiotic combinations according to current definitions of synergism. The researchers used 17 MRSA strains to evaluate vancomycin-gentamicin synergism. Under the synergism definition of a 100-fold increase in killing for the combination compared with the most active single agent at 24 h, only 3 of the 17 strains were synergistically killed; only 5 of the 17 strains were synergistically killed if a less stringent criterion of a 10-fold increase in killing at 24 h is used. For the MRSA isolates tested in that study, the median gentamicin MIC was 3.12 µg/ml and the range was 0.78 to >25 µg/ml. The results were similar for tobramycin. The researchers stated that there was “no difference” between gentamicin-resistant and -susceptible strains, but data correlating the gentamicin MIC with synergism were not provided.

In our study, we screened and categorized our MRSA strains according to gentamicin susceptibility by using the same criteria for high-level aminoglycoside resistance as defined for enterococci. As is the case for enterococci, none of the MRSA isolates with an agar dilution gentamicin MIC of >500 µg/ml showed vancomycin-gentamicin synergism. However, unlike for enterococci, for most of the MRSA isolates for which the gentamicin MICs were <500 µg/ml there was no evidence of synergistic killing. Synergism could be demonstrated for only 6 of 15 such strains. Synergism was not predictable by the agar dilution gentamicin MIC; for six resistant strains (MIC >4 µg/ml), synergism was demonstrated, and for two susceptible strains, indifference was demonstrated.

Although combination therapy is recommended by some experts for the treatment of deep-seated MRSA infections such as prosthetic valve endocarditis (5), there are no controlled trials demonstrating the superiority of the combination over vancomycin alone. As the combination of vancomycin and gentamicin is more nephrotoxic than gentamicin alone (2, 3), one may reasonably question the addition of an aminoglycoside to vancomycin unless there is some expectation of a benefit. Definitive guidelines for the use of vancomycin-gentamicin combination therapy for MRSA infections await the completion of clinical trials correlating outcome with in vitro synergy studies.

REFERENCES