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In Vitro Killing of Community-Associated Methicillin-Resistant *Staphylococcus aureus* with Drug Combinations

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This study employs time-kill techniques to examine the most common drug combinations used in the therapy of methicillin-resistant *Staphylococcus aureus* (MRSA) infections, vancomycin plus either gentamicin or rifampin. Community-associated MRSA were more likely to be synergistically inhibited by combinations of vancomycin and gentamicin versus vancomycin alone compared to inhibition associated with hospital-acquired strains.

Despite being considered the drug of choice for serious methicillin-resistant *Staphylococcus aureus* (MRSA) infections, vancomycin therapy for MRSA often yields less than ideal results compared to that of β-lactam treatment of methicillin-susceptible *S. aureus* (MSSA) (6, 7, 19, 22, 28). The suboptimal response of MRSA infections to vancomycin often leads clinicians to add a second or even third antimicrobial, the most widely used of which have been rifampin and aminoglycosides (5, 15), despite discordant in vitro studies and the lack of supporting clinical data (13, 21, 27). Over the past several years, there has been a dramatic rise in the isolation of MRSA from patients who have no recognized link to the hospital (2, 10, 24). Community-associated (CA)-MRSA strains are often sensitive to a much broader array of antimicrobials other than β-lactams than are hospital-acquired (HA)-MRSA strains (23, 29).

We hypothesized that the greater susceptibility of CA-MRSA to gentamicin and rifampin would result in increased bactericidal activity, compared to that of HA-MRSA, when these drugs were combined with vancomycin. The purposes of this investigation were (i) to study the differential effects of combination therapy of vancomycin plus gentamicin or vancomycin plus rifampin versus vancomycin alone in CA-MRSA in comparison with HA-MRSA and MSSA and (ii) to evaluate whether altering the dose of rifampin changed the in vitro results of the combination of vancomycin and rifampin.

Unique patient isolates of *S. aureus* were selected to obtain 25 CA-MRSA, 10 HA-MRSA, and 11 MSSA. Designation of isolates as CA versus HA was made in accordance with Centers for Disease Control and Prevention guidelines (14). MICs were determined by disk diffusion methods and by broth macrodilution technique. Time-kill studies were done following the methods of Eliopoulos and Mollering (11), with a starting inoculum of 5×10^6 CFU/ml. Vancomycin was studied at 10 μg/ml, gentamicin at 1 μg/ml, and rifampin at 0.5 μg/ml; selected strains were also studied at rifampin concentrations of 0.016 and 3 μg/ml. For the purposes of this study, synergy was said to be present if, after a 24-h incubation, the number of CFU was ≥ 2 $\log_{10}$ lower in the presence of the combination than with the single, more active, agent. Antagonism was said to be present if CFU were ≥ 2 $\log_{10}$ higher after incubation with the combination than with the single, more active, agent.

All strains of *S. aureus* used in this study were susceptible to vancomycin, with no differences among CA-MRSA, HA-MRSA, or MSSA. CA-MRSA were more likely to be susceptible to gentamicin (90%) than were HA-MRSA (50%) ($P = 0.016$). The MIC of rifampin ranged from ≤ 0.016 to 0.25 μg/ml, with a minimum 50% inhibitory concentration of 0.31 μg/ml with no differences among the three groups of organisms.

The mean rate of killing for vancomycin alone was similar among HA-MRSA, CA-MRSA, and MSSA (at 24 h, $P = 0.877$; Table 1). In the presence of 1 μg of gentamicin/ml alone, CFU for all isolates declined by a mean of 0.79 $\log_{10}$ at 24 h. When the two drugs were added together, killing was nearly 100-fold greater or more at each time point than it had been for vancomycin alone (Table 1, Fig. 1a). Synergy was observed for 23 out of 25 (92%) CA-MRSA, 5 out of 10 (50%) HA-MRSA, and 8 out of 11 (73%) MSSA (synergy was more frequent for CA-MRSA and MSSA than for HA-MRSA [$P = 0.022$]; CA-MRSA were not different from MSSA [$P = 0.123$]). At 24 h, the mean decrease with the combination of vancomycin and gentamicin compared to that with vancomycin alone was 2.78 $\log_{10}$ for CA-MRSA, 2.84 $\log_{10}$ for MSSA, and 1.49 $\log_{10}$ for HA-MRSA ($P = 0.025$ for CA-MRSA and $P = 0.023$ for MSSA, having greater decreases versus HA-MRSA; Fig. 1b). Synergy was noted for 41% of gentamicin-resistant isolates and 91% of susceptible isolates ($P < 0.001$).

In contrast, the addition of rifampin at 0.5 μg/ml to vancomycin resulted in decreased bactericidal activity at 4, 8, and 24 h compared to the activity of vancomycin alone (Table 1, Fig. 1a), an effect that was similar among the three groups of isolates ($P = 0.734$). No evidence of synergistic effect was noted for any of the isolates, whereas the presence of rifampin

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TABLE 1. Time-kill responses for *S. aureus*

Organisms and drug(s)[a]	Mean CFU $\log_{10}$ decrease at:			Mean CFU $\log_{10}$ difference compared to that of vancomycin alone at 24 h	*P* value for combination compared to that of vancomycin alone at 24 h
	4 h	8 h	24 h		
All *S. aureus* isolates					
Vancomycin alone	−0.28	−0.65	−2.04		
Vancomycin + rifampin	−0.20	−0.31	−0.62	+1.42	<0.001
Vancomycin + gentamicin	−2.09	−3.15	−4.54	−2.49	<0.001
All MRSA					
Vancomycin alone	−0.33	−0.64	−2.06		
Vancomycin + rifampin	−0.25	−0.32	−0.64	+1.42	<0.001
Vancomycin + gentamicin	−1.95	−3.03	−4.47	−2.41	<0.001
HA-MRSA					
Vancomycin alone	−0.26	−0.54	−2.21		
Vancomycin + rifampin	−0.02	−0.20	−0.53	+1.68	<0.001
Vancomycin + gentamicin	−1.44	−2.24	−3.71	−1.49	0.005
CA-MRSA					
Vancomycin alone	−0.31	−0.68	−1.95		
Vancomycin + rifampin	−0.35	−0.38	−0.69	+1.26	<0.001
Vancomycin + gentamicin	−2.24	−3.47	−4.73	−2.78	<0.001
MSSA					
Vancomycin alone	−0.30	−0.75	−2.00		
Vancomycin + rifampin	−0.04	−0.28	−0.54	+1.45	<0.001
Vancomycin + gentamicin	−2.11	−3.22	−4.84	−2.84	<0.001

[a] Rifampin dose, 0.5 μg/ml; gentamicin dose, 1 μg/ml.

was antagonistic in nine strains (four CA-MRSA strains, three HA-MRSA strains, and two MSSA strains). Similar results were obtained when rifampin was studied at either low dose (0.16 μg/ml) or high dose (3.0 μg/ml) (data not shown).

One of the characteristics of CA-MRSA are their propensity to carry the basis for methicillin resistance, the *mec* gene, in a novel type of staphylococcal chromosomal cassette (SCC) known as type IV SCC*mec* (9, 17). The SCC*mec* type of each MRSA isolates was determined by PCR using the method of Okuma et al. (26). Positive controls NCTC 10492, (SCC*mec* type I), N315 (SCCmec type II), 85/2082 (SCC*mec* type III), and CA 05 (SCC*mec* type IVa) were kindly provided by Keiichi Hiramatsu and Teruyo Ito, Department of Bacteriology, Juntendo University, Tokyo, Japan. All of the isolates studied here that were classified as CA by epidemiologic guidelines published by the Centers for Disease Control and Prevention carried type IVa SCC*mec*. Five of the HA-MRSA had type II SCC*mec* while the other five contained type IVa. No strains carried type I or type III SCC*mec*. The CA-MRSA were more likely to carry type IV SCC*mec* than were hospital isolates ($P = 0.001$). The MRSA isolates containing type IVa SCC*mec* were more likely to be synergistically affected by the combination of vancomycin and gentamicin than were those having type II SCC*mec* ($P = 0.044$).

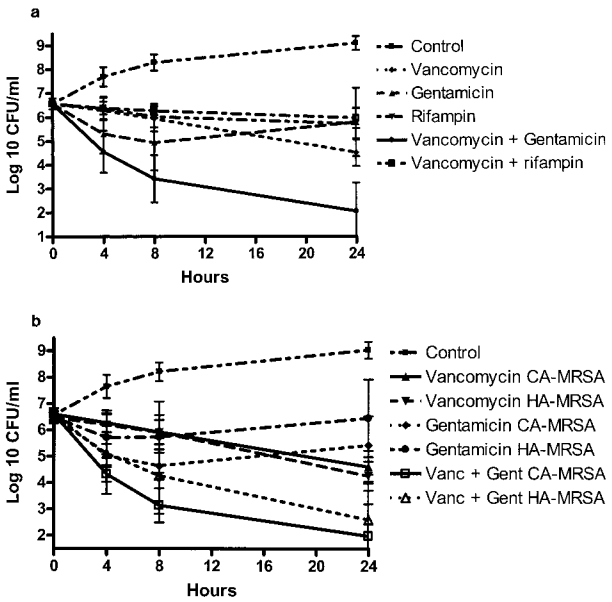

FIG. 1. (a) Time-kill curves for all isolates of *S. aureus*. Antibiotic concentrations: vancomycin, 10 μg/ml; gentamicin, 1 μg/ml; rifampin, 0.625 μg/ml. Errors bars indicate ±1 standard deviation. (b) Time-kill curves for isolates of MRSA. Error bars indicate ±1 standard deviation.

Treatment of serious MRSA infections with vancomycin is associated with persistent bacteremia and a substantial rate of complication and relapse (7, 19). Until recently, MRSA infections were nearly always HA, and the known tendency of these organisms to exhibit aminoglycoside resistance reduced interest in adding gentamicin to vancomycin (3). The finding that nearly all CA-MRSA studied here were sensitive to gentamicin is in accord with previously published reports (1, 10). An unexpected finding, however, was that 5 of 10 HA-MRSA strains also were sensitive to gentamicin. Aminoglycoside testing of MRSA in the past has suggested much higher rates of resistance (3). The results of this study suggest that aminoglycoside

resistance among MRSA at our center is decreasing in a manner similar to that recently reported in France and in Minnesota (18, 23).

The decreased killing activity of *S. aureus* when rifampin was added to vancomycin is in accordance with previous reports that employed time-kill methods (16, 31, 32). The significance of in vitro studies with this combination has been questioned by some authors, who have noted indifference or antagonism when test tube methods were used but appear to have shown synergy in animal models (4, 25, 27). The clinical evidence for the use of this combination is equally discordant, with one trial and a few case reports reporting efficacy in treating MRSA infections while in a randomized trial the addition of rifampin to vancomycin for MRSA bacteremia had no effect (12, 19, 20, 30). However, previous in vitro data has suggested that the addition of rifampin to cell wall-active agents may facilitate bactericidal activity, particularly against organisms sequestered within foci that are not readily accessible to antibiotics or immune mechanisms (8). Another previous study noted that rifampin was synergistic with ciprofloxacin at sub-MIC doses but was antagonistic at higher doses (31). When varying the concentration of rifampin between 0.0156 μg/ml (sub-MIC) up to 3.0 μg/ml, we found no difference in the killing effects of the combination of vancomycin and rifampin. Thus, the antagonistic interaction noted in vitro between vancomycin and rifampin is not concentration dependent.

The slow response of some MRSA infections to vancomycin is likely to prompt clinicians to continue to employ additional agents in combination with vancomycin in an attempt to improve the response to therapy. There appears to be changing resistance patterns to aminoglycosides among MRSA isolates that might be expected to make these organisms more responsive to combination therapy. At the present time, the high rates of gentamicin susceptibility among CA-MRSA suggest that combination therapy may be particularly effective for patients with infections due to these isolates. Clinical studies evaluating this regimen are needed more than ever given the rising rates of methicillin resistance among community *S. aureus* isolates.

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