Activity of Daptomycin Alone and in Combination with Rifampin and Gentamicin against Staphylococcus aureus Assessed by Time-Kill Methodology††

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The synergistic effects of daptomycin plus gentamicin or rifampin were tested against 50 Staphylococcus aureus strains, with daptomycin MICs ranging between 0.25 and 8 µg/ml. Daptomycin sub-MICs combined with gentamicin concentrations lower than the MIC yielded synergy in 34 (68%) of the 50 strains. Daptomycin combined with rifampin yielded synergy in one vancomycin-intermediate S. aureus strain only, and virtually all synergy occurred between daptomycin and gentamicin.

Methicillin-resistant Staphylococcus aureus (MRSA) strains are increasingly encountered and cannot be treated with available β-lactams. Most methicillin-resistant (and also some methicillin-susceptible S. aureus [MSSA]) strains are resistant to all available quinolones, and vancomycin-intermediate (VISA) and vancomycin-resistant (VRSA) S. aureus strains have appeared (3–5, 8, 11, 15, 23). Most multidrug-resistant S. aureus strains are nosocomially acquired and cause an array of site-specific infections in hospitalized patients, including bloodstream infections, pneumonia, surgical-site infections, and urinary tract infections. However, in the past few years there has been an increase in the incidence of community-acquired MRSA which, although at this time susceptible to most other agents, are more virulent than hospital strains (1, 7, 10, 12–14, 18, 21, 22). Although previously considered to play an important role in the virulence of community-acquired MRSA strains, a recent report (25) has cast doubt upon the importance of Panton-Valentine leukocidin production in the pathogenicity of these strains.

The development of S. aureus strains with diminished susceptibility to vancomycin is at least partially caused by the selective pressure of vancomycin use in the community (23). The increase in community-acquired MRSA strains will likely lead to more glycopeptide use in the community setting, therefore increasing the selective pressure for vancomycin resistance. An alternative to glycopeptides is urgently needed.

Daptomycin is very potent against S. aureus, with low MICs, rapid killing, and excellent clinical activity (17, 20). Daptomycin is currently approved in the United States for the treatment of skin and soft tissue infections, S. aureus bacteremia, and right-sided endocarditis (20). Increasing daptomycin MICs have been reported and a possible correlation (based perhaps on the shared activity of both drugs at different sites in a thickened cell envelope) for higher daptomycin MICs in laboratory derived-serial passage isolates has been described (3, 4).

We have used time-kill synergy studies to assess the activity of daptomycin, alone and combination with rifampin and gentamicin, against 50 S. aureus strains with various daptomycin MICs.

The S. aureus isolates included the following: VISA, 6 strains; VRSA, 3 strains; MSSA, 9 strains; and MRSA, 32 strains (20 community-acquired, 15 of these were Panton-Valentine leukocidin positive, and 12 were nosocomially acquired). Strains were isolated from the Hershey Medical Center and the University of Texas Southwestern Medical Center, Dallas, TX. Strains were stored frozen at −70°C in double-strength skim milk (Difco, Inc., Detroit, MI) before testing. Daptomycin was obtained from Cubist Pharmaceuticals, Lexington, MA, and rifampin and gentamicin were from Sigma Chemical Co., Inc., St. Louis, MO.

MICs were predetermined by macrodilution in cation-adjusted Mueller-Hinton broth (BBL Microbiology Systems, Cockeysville, MD) according to standard methodology (19). Daptomycin susceptibility testing was performed in Mueller-Hinton broth adjusted to 50 µg/ml of calcium according to standard methodology. All strains were tested by time-kill methodology with each compound alone as described previously (2). Concentrations (35-µl aliquots of suspensions into 5 ml of broth) at one to two dilutions below the MIC were chosen for synergy testing. Viability counts (100-µl aliquots) in synergy tests were performed at 0, 3, 6, 12, and 24 h in a shaking water bath at 35°C with final inocula of between 5 × 105 and 5 × 106 CFU/ml. Only plates with 30 to 300 colonies were counted. Drug carryover was addressed by dilution as described previously. Synergy was defined as a 2 log10 decrease in CFU/ml between the combination and its most active component after 3, 6, 12, and 24 h and the number of surviving organisms in the presence of the combination being ≥2 log10 below the starting inoculum at 0 h. At least one of the drugs had to be present in a concentration which did not significantly affect the growth curve of the test organism when used alone (2).

Results of our study correlated by the strain’s resistotype are presented in Table 1. Individual synergy data are avail-

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able electronically in the supplemental material. The MICs of the drugs alone were 0.25 to 8 μg/ml (daptomycin), 0.04 to >128 μg/ml (rifampin), and 0.5 to 1,024 μg/ml (gentamicin). Six VISA strains had increased daptomycin MICs of 2 to 8 μg/ml. Six strains with rifampin MICs of ≥64 μg/ml and 12 strains with gentamicin MICs of >128 μg/ml were not tested.

Only one VISA isolate showed synergy between daptomycin and rifampin at both 12 h (2 and 0.004 μg/ml) and 24 h (2 and 0.008 μg/ml); all other combinations were additive. At 3 h, three strains (one MSSA and two community-acquired MRSA isolates, toxin positive) showed synergy between daptomycin and gentamicin (two strains, 0.125 and 0.5 μg/ml; one strain, 0.25 and 0.25 μg/ml). At 6 h, six community-acquired MRSA toxin-positive strains showed synergy at 0.125 and 0.5 μg/ml; in addition, two MSSA strains showed synergy at 0.125 and 0.25 μg/ml; one VISA strain showed synergy at 1 and 0.5 μg/ml; one VRSA strain showed synergy at 0.06 and 32 μg/ml. At 12 h, synergy was seen in 8 MSSA strains (0.125 and 0.25 μg/ml), 13 community-acquired MRSA strains (11 toxin positive; 0.125 and 0.5 μg/ml); 4 hospital-acquired MRSA strains (0.25 and 0.25 μg/ml), 3 VISA strains (1 and 0.5 μg/ml), and three VRSA strains (0.06 and 32 μg/ml). Synergy was seen at 24 h in 4 MSSA strains (0.25 and 0.125 μg/ml), 13 community-acquired MRSA strains (10 toxin positive) (0.125 and 1 μg/ml), 3 hospital-acquired MRSA strains (0.25 and 0.5 μg/ml), 2 VISA strains (2 and 0.5 μg/ml), and 2 VRSA strains (0.06 and 32 μg/ml). All other combinations with daptomycin and gentamicin at all time periods were additive, and no antagonism was found.

Of the nine MSSA strains tested 12% (one of eight strains), 25% (two of eight strains), 100% (all eight strains), and 50% (four of eight strains) showed synergy with daptomycin and gentamicin at 3, 6, 12, and 24 h, respectively. Of the 20 community-acquired MRSA strains tested 11% (2 of 19 strains), 32% (6 of 19 strains) at both 3 and 6 h, and 68% (13 of 19 strains) at both 12 and 24 h showed synergy with daptomycin and gentamicin at sub-MIC combinations. Synergy was also found in 80% (four of five strains) and 60% (three of five strains) at both 12 and 24 h in 12 hospital-acquired MRSA with daptomycin plus gentamicin. The six VISA strains demonstrated synergy with daptomycin and gentamicin with 67% (two of three strains) at both 6 and 24 h and 100% (three of three strains) at 12 h. One of the three VISA strains (33%) showed synergy with daptomycin plus rifampin at both 12 and 24 h. Of the three VRSA strains tested with daptomycin plus gentamicin 33% (one of three strains), 100% (three of three strains), and 67% (two of three strains) showed synergy at 6, 12, and 24 h, respectively.

Synergy time-kill graphs for one VISA strain and one community-acquired MRSA strain are depicted graphically in Fig. 1.

Laplante and Rybak (16) evaluated the impact of high-inoculum Staphylococcus aureus (9.5 log_{10} CFU/g) on the activities of daptomycin, alone and in combination with gentamicin in an in vitro pharmacodynamic model with simulated endocardial vegetations over 72 h. In both strains tested (one MSSA strain and one MRSA strain), the addition of gentamicin increased the rate of 99.9% kill to 8 h for daptomycin (P < 0.01). Tsuji and Rybak (24) have reported that a single dose of gentamicin (5 mg/kg) in combination with daptomycin may be of use to maximize synergistic and bactericidal activity and minimize toxicity, in an in vitro pharmacodynamic model. In contrast, a recent study by DeRyke et al. (9) showed that the coadministration of gentamicin did not alter daptomycin pharmacokinetics. Daptomycin retained bactericidal activity in the presence of gentamicin against most strains except for Enterococcus faecium. No instances of antagonism were observed. Results of combinations between daptomycin and other aminoglycosides have not, to our knowledge, been reported, and the toxicity of both combinations at sub-MICs is not expected to increase. The daptomycin C_{max} for skin and soft tissue infections at an approved dose of 4 mg/kg is 57.8 μg/ml and that for bacteremia–right-sided endocarditis at a dose of 6 mg/kg is 98.6 μg/ml (20). At a gentamicin dose of 2 mg/kg the C_{max} values in healthy volunteers were 10.1 ± 1.3 μg/ml (predistribution peak) and 6.2 ± 0.4 μg/ml (postdistribution peak), and at a gentamicin dose of 7 mg/kg the C_{max} values in healthy volunteers were 39.8 ± 4.1 μg/ml (predistribution peak) and 11.0 ± 0.6 μg/ml (postdistribution peak) (6). More work is necessary before the clinical utility of the current findings can be fully assessed. Although synergy time-kill studies are be-

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**TABLE 1. Combined results of MIC and time-kill synergy tests**

<table>
<thead>
<tr>
<th>Strain</th>
<th>No. of strains</th>
<th>MIC range (μg/ml)</th>
<th>% of strains</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>DAP</td>
<td>RIF</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Ant</td>
<td>Add</td>
</tr>
<tr>
<td>MSSA</td>
<td>9</td>
<td>0.25–1</td>
<td>0.008–0.016</td>
</tr>
<tr>
<td>CA-MRSA, PVL*</td>
<td>15</td>
<td>0.5–1</td>
<td>0.004–0.016</td>
</tr>
<tr>
<td>CA-MRSA, PVL*</td>
<td>5</td>
<td>0.5–1</td>
<td>0.016–&gt;64</td>
</tr>
<tr>
<td>HA-MRSA</td>
<td>12</td>
<td>0.25–1</td>
<td>0.004–&gt;256</td>
</tr>
<tr>
<td>VISA</td>
<td>6</td>
<td>2–8</td>
<td>0.016–&gt;128</td>
</tr>
<tr>
<td>VRSA</td>
<td>3</td>
<td>0.25–1</td>
<td>0.008–&gt;128</td>
</tr>
</tbody>
</table>

a DAP, daptomycin; RIF, rifampin; GEN, gentamicin.
b That is, the percentage of strains at the 24-h time point demonstrating the labeled effect. Ant, antagonistic; Add, additive; Syn, synergistic.
c Isolate 105 was not tested with the DAP + GEN combination.
d Isolate 347 was not tested with the DAP + RIF or the DAP + GEN combination.
e Isolates 076, 081, 086, 251, 243, and 181 were not tested with the DAP + GEN combination, and isolate 069 was not tested with either combination.
f Isolates 555 and 506 were not tested with the DAP + RIF combination, isolates 507 and 508 were not tested with the DAP + GEN combination, and isolate 504 was not tested with either combination.
g Isolate 509 was not tested with the DAP + RIF combination.
Beyond the capability of most routine laboratories, the Etest method (AB Biodisk, Solna, Sweden) may be used for this purpose.

Although daptomycin is uniformly active, as well as rapidly bactericidal, against the vast majority of *S. aureus* strains encountered clinically (17, 20), recent studies (3, 4) point to a slightly higher daptomycin MIC for some VISA strains, and it is speculated that this may be because of abnormalities in the cell envelope. The present study shows that at 6, 12, or 24 h significant synergy was obtained in vitro between sub-MIC concentrations of daptomycin and gentamicin. Clinical studies will be required to test this hypothesis.

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### REFERENCES


