ACTIVITY OF DAPTOMYCIN ALONE AND IN COMBINATION WITH RIFAMPIN AND GENTAMICIN AGAINST *STAPHYLOCOCCUS AUREUS* BY TIME-KILL METHODOLOGY

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Running title: Daptomycin staphylococcal synergy

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ABSTRACT. Synergistic effects of daptomycin plus gentamicin or rifampin were tested against 50 *S.aureus* strains with daptomycin MICs ranging between 0.25-8µg/ml. SubMIC daptomycin concentrations 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 VISA 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) strains are resistant to all available quinolones and vancomycin intermediate (VISA) and vancomycin fully 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 blood stream 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 (PVL) production in the pathogenicity of these strains.

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 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 USA for the treatment of skin and soft tissue infection, *S. aureus* bacteremia, and right-sided endocarditis (20). Increasing daptomycin MICs have been reported and a possible correlation, (based perhaps on shared activity of both drugs on 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 varying daptomycin MICs.

The S. aureus isolates were as follows: VISA: 6 strains; VRSA: 3 strains; methicillin-susceptible: 9 strains; methicillin-resistant: 32 strains (20 community-acquired, 15 of these PVL positive, and 12 nosocomially acquired. Strains were isolated from Hershey Medical Center and University of Texas Southwestern Medical Center, Dallas, TX. Strains were stored frozen at -70°C in double strength litmus milk (Difco, Inc., Detroit, Mi) before testing. Daptomycin was obtained from Cubist Pharmaceuticals, Lexington, MA and rifampin and gentamicin 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 in MH-broth adjusted to 50 µg/ml of calcium per standard methodology. All strains were tested by time-kill with each compound alone as described previously (2). Concentrations (35 µl aliquots of suspensions into 5 ml broth) at 1-2 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 between 5 x 10^5 and 5 x 10^6 cfu/ml. Only plates with 300-300 colonies were counted. Drug carryover was addressed by dilution as described previously. Synergy was defined as a ≥2log_{10} 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 $\geq 2\log_{10}$ 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 available electronically as supplementary data. MICs (µg/ml) of drugs alone were 0.25-8 (daptomycin), 0.004-$\geq$128 (rifampin), 0.5-1024 (gentamicin). Six VISA strains had raised daptomycin MICs (2-8 µg/ml). Six strains with rifampin MICs $\geq$64 µg/ml and 12 with gentamicin MICs $>128$ µg/ml were not tested.

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

Of the 9 methicillin susceptible stains tested 12% (1/8 strains), 25% (2/8 strains), 100% (8/8 strains) and 50% (4/8 strains) showed synergy with daptomycin + gentamicin at 3, 6, 12, and 24 h respectively. Of the 20 community-acquired MRSA strains tested 11% (2/19 strains), 32% (6/19 strains) at both 3 and 6 h and 68% (13/19 strains) at both 12 and 24 h showed synergy with daptomycin + gentamicin at subMIC combinations. Synergy was also found in 80% (4/5 strains) and 60% (3/5 strains) at both 12 and 24 h in 12 hospital-acquired MRSA with daptomycin + gentamicin. The 6 VISA strains demonstrated synergy with daptomycin + gentamicin with 67% (2/3 strains) at both 6 and 24 h and 100% (3/3 strains) at 12 h. One of the 3 VISA strains (33%) showed synergy with daptomycin + rifampin at both 12 and 24 h. Of the 3 VRSA strains tested with daptomycin + gentamicin 33% (1/3 strains), 100% (3/3 strains), and 67% (2/3 strains) showed synergy at 6, 12, and 24 h, respectively.

Synergy time-kill graphs for one VISA and one community acquired-MRSA are depicted graphically in Figures 1 and 2.

Laplante and Rybak (16) evaluated impact of high-inoculum Staphylococcus aureus (9.5 $\log_{10}$ cfu/g) on 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 (1 methicillin-susceptible, 1 MRSA), 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. By contrast, a recent paper by DeRyke et al. (9) showed that co-administration of gentamicin did not alter daptomycin pharmacokinetics. Daptomycin retained bactericidal activity in 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 toxicity of both combinations at subMIC concentrations is not expected to increase. The daptomycin $C_{\text{max}}$ 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 gentamicin doses of 2 mg/kg and 7 mg/kg, $C_{\text{max}}$ values (µg/ml) in healthy volunteers were: 10.1 ± 1.3 (predistribution peak) and 6.2 ± 0.4 (post-distribution peak), and 39.8 ± 4.1 (predistribution peak) and 11.0 ± 0.6 (postdistributional peak), respectively (6). More work is necessary before clinical utility of the current findings can be fully assessed.

Although synergy time-kills are beyond the capability of most routine laboratories, the E test 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 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 current study shows that, at 6 h, 12 h or 24 h, significant synergy was obtained in vitro between subMIC concentrations of daptomycin and gentamicin. Clinical studies will be required to test this hypothesis.
This study was supported by a grant from Cubist Pharmaceuticals, Lexington, MA. We thank George McCracken and Susanna Chavez-Bueno for kind provision of some of the strains tested in this study.
REFERENCES


5. De Lassence, A, N. Hidri, J.-F. Timsit, M.-L. Joly-Guillou, G. Thiery, A. Boyer, P. Lable, A. Blivet, H. Kalinowski, Y. Martin, J.-P. Lajonchere, and D. Dreyfuss. Control and outcome of a large outbreak of colonization and infection with glycopeptide-


Table 1. Combined results of MIC and tine-kill synergy tests.

<table>
<thead>
<tr>
<th>Strain</th>
<th>N</th>
<th>DAP MIC Range</th>
<th>RIF MIC Range</th>
<th>GENT MIC Range</th>
<th>DAP + RIF</th>
<th>DAP + GENT</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>% Antagonistic</td>
<td>% Additive</td>
<td>% Synergistic</td>
<td>% Antagonistic</td>
<td>% Additive</td>
</tr>
<tr>
<td>MSSA</td>
<td>9</td>
<td>0.25-1</td>
<td>0.008-0.016</td>
<td>0.5-&gt;128</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>CA-MRSA, PVL+</td>
<td>15</td>
<td>0.5-1</td>
<td>0.004-0.016</td>
<td>1-2</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>CA-MRSA, PVL-</td>
<td>5</td>
<td>0.5-1</td>
<td>0.016-&gt;64</td>
<td>1-&gt;128</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>HA-MRSA</td>
<td>12</td>
<td>0.25-1</td>
<td>0.004-256</td>
<td>1-512</td>
<td>0%</td>
<td>100%</td>
</tr>
<tr>
<td>VISA</td>
<td>6</td>
<td>2-8</td>
<td>0.016-&gt;128</td>
<td>1-1024</td>
<td>0%</td>
<td>66.7%</td>
</tr>
<tr>
<td>VRSA</td>
<td>3</td>
<td>0.25-1</td>
<td>0.008-&gt;128</td>
<td>64-128</td>
<td>0%</td>
<td>100%</td>
</tr>
</tbody>
</table>

- Percentage of strains at the 24 hr time point demonstrating the labeled effect.
- Isolate 105 was not tested with the DAP+GENT combination.
- Isolate 347 was not tested with the DAP+RIF or DAP+GENT combination.
- Isolates 076, 081, 086, 251, 243, 181 were not tested with the DAP+RIF combination and 069 was not tested with either combination.
- Isolates 555, 506 were not tested with the DAP+RIF combination, 507 and 508 were not tested with the DAP+GENT combination, and 504 was not tested with either combination.
- Isolate 509 was not tested with the DAP+RIF combination.
Legends for Figures 1 and 2.

In vitro activities of daptomycin and gentamicin, alone and combined, against 1 MRSA and 1 VISA strain. For methods see above.