Bactericidal Activities of Daptomycin, Quinupristin-Dalfopristin, and Linezolid against Vancomycin-Resistant *Staphylococcus aureus* in an In Vitro Pharmacodynamic Model with Simulated Endocardial Vegetations

Raymond Cha,1,2 William J. Brown,2,3 and Michael J. Rybak1,2,4,*

Anti-Infective Research Laboratory, Eugene Applebaum College of Pharmacy and Health Sciences,1 School of Medicine,2 and DMC University Laboratories,3 Detroit Receiving Hospital,4 Wayne State University, Detroit, Michigan 48201

Received 25 February 2003/Returned for modification 17 April 2003/Accepted 25 August 2003

Grave concerns regarding gram-positive resistance were recently amplified with the first two reports of infections due to vancomycin-resistant *Staphylococcus aureus* (VRSA) (8, 9). The possibility of further identification of infections due to VRSA and the difficult complications associated with this pathogen (i.e., endocarditis) emphasize the need for evaluation of antimicrobial agents that possess bactericidal activity in the presence of high inoculum, protein, and antibiotic penetration barriers.


Two clinical strains of *S. aureus*, including the reported VRSA isolate (DMC83006A) and an earlier vancomycin-sensitive, methicillin-resistant *S. aureus* (MRSA) isolate (DMC82991; the presumptive VRSA parent) from the same patient were evaluated at the Department of Microbiology, DMC University Laboratories, Wayne State University, Detroit, Mich. (9).

Microdilutional MICs and minimum bactericidal concentrations (MBCs) were determined pre- and postexposure according to NCCLS guidelines, and E-test methods were employed for confirmation of results (22). In addition, daptomycin MIC and MBC analyses were performed in the presence of human albumin (American Red Cross, Detroit, Mich.) at 4 g/dl (20, 25).

Mueller-Hinton broth (Becton-Dickinson, St. Louis, Mo.) supplemented with 25 mg of calcium per liter and 12.5 mg of magnesium per liter was used for experiments with vancomycin, quinupristin, dalfopristin, and linezolid. Mueller-Hinton broth supplemented with 75 mg of calcium per liter and 12.5 mg of magnesium per liter was used for daptomycin experiments due to its dependence on calcium for activity (16, 19). E-test MICs for quinupristin-dalfopristin, vancomycin, and linezolid were determined by using tryptic soy agar (TSA; Becton-Dickinson) plates. IsoSensitest agar (Oxoid, Inc., Ogdensburg, N.Y.) was used for daptomycin E tests.

As previously described, an in vitro pharmacodynamic model with simulated endocardial vegetations was utilized (1, 17). The following regimens were simulated: daptomycin, 6 mg/kg of body weight every 24 h (peak, 98.6 μg/ml; average half-life, 8 h); quinupristin-dalfopristin, 7.5 mg/kg every 8 h (quinupristin [peak, 3 μg/ml; average half-life, 1 h] and dalfopristin [peak, 8 μg/ml; average half-life, 0.7 h]); linezolid, 600 mg every 12 h (peak, 18 μg/ml; average half-life, 5 h), and vancomycin, 1 g every 12 h (peak, 40 μg/ml; average half-life, 6 h). For quinupristin-dalfopristin simulations, each component was administered separately in order to facilitate the simulation of their respective half-lives by setting the elimination at the shorter half-life and supplementing the agent with the longer half-life (6). All in vitro pharmacodynamic experiments were performed in triplicate. In addition, growth control conditions were tested.

### Table 1.Susceptibility results (MIC/MBC in mg/liter)

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>VRSA (DMC83006A)</th>
<th>MRSA (DMC82991)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Daptomycin</td>
<td>0.25/0.50</td>
<td>0.125/0.25</td>
</tr>
<tr>
<td>Daptomycin (albumin)</td>
<td>1.0/4.0</td>
<td>1.0/2.0</td>
</tr>
<tr>
<td>Linezolid</td>
<td>2.0/32</td>
<td>2.0/16</td>
</tr>
<tr>
<td>Quinupristin-dalfopristin</td>
<td>0.25/0.50</td>
<td>0.25/0.50</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>1.02/2.048</td>
<td>1.0/2.0</td>
</tr>
</tbody>
</table>

* Corresponding author. Mailing address: Anti-Infective Research Laboratory, Pharmacy Practice—4148, Eugene Applebaum College of Pharmacy and Health Sciences, Wayne State University, 259 Mack Ave., Detroit, MI 48201. Phone: (313) 993-4673. Fax: (313) 577-8915. E-mail: m.rybak@wayne.edu.
Three simulated endocardial vegetations were removed from each model (total, nine for each drug regimen experiment at each time point) at 0, 8, 24, 32, 48, and 72 h. Simulated endocardial vegetations were then homogenized and diluted as necessary (10- to 100,000-fold) into sterilized cold 0.9% sodium chloride solution. Aliquots of all dilutions were then plated onto TSA in triplicate and incubated at 35°C for 24 h. This method results in a lower limit of detection of 2.0 log_{10} CFU/g. Antimicrobial carryover was minimized by serial dilution of plated samples in conjunction with gravity filtration. Pharmacodynamic profiles were constructed by plotting time-kill curves in log_{10} CFU/g over 72 h. Bactericidal activity (99.9% kill) was defined as a ≥3-log_{10} CFU/g reduction in colony count from the initial inoculum. Bacteriostatic activity was defined as a 0.5- to 2.99-log_{10} CFU/g reduction in colony count from the initial inoculum, while inactivity was defined as...
exhibiting no observed reductions. The time to achieve a 99.9% (T99) bacterial load reduction was determined by subtracting density of samples from the initial inoculum and identifying the first time point for which a 99.9% kill occurred.

Pharmacokinetic samples from the central compartment were obtained at peak (2 min after the end of antimicrobial administration) and at 1, 2, 4, 8, 24, 32, 48, and 72 h for verification of target concentrations (17). Vancomycin concentrations were determined by fluorescence polarization immunoassay (Abbott Diagnostics TDX). Concentrations of daptomycin were determined by a previously described microbioassay (15). Quinupristin and dalfopristin concentrations were determined separately by a previously reported microbioassay (15). Linezolid concentrations were determined by a previously described validated high-pressure liquid chromatography assay (4). A one-compartment model with bolus intravenous input and first-order elimination was applied to concentration data with PK Analyst software (Micromath Research, St. Louis, Mo.) to determine elimination rates and free peak and trough concentrations.

Development of resistance was evaluated at 24, 48, and 72 h by plating 100 μl of each sample onto TSA plates containing four and eight times the MIC of the respective antimicrobial agent. Plates were then examined for growth after 48 h of incubation at 35°C.

Changes in the number of CFU per gram at 24, 48, and 72 h were compared by one-way analysis of variance with Tukey’s post hoc test. A P value of ≤ 0.05 was considered significant. All statistical analyses were performed using SPSS statistical software (version 10.07; SPSS, Inc., Chicago, Ill.).

Susceptibility results are presented in Table 1. Pharmacodynamic results (change in log CFU per gram over 72 h) are presented in Fig. 1. For all simulations, pH ranged between 6.98 and 7.14 and the temperature was maintained at 37°C. Initial inocula for all regimen simulations were within 0.5 log10 CFU/g of the target inoculum. All simulations achieved peak concentrations, and half-lives were within 10% of targeted values. Daptomycin and quinupristin-dalfopristin achieved rapid bactericidal activity, with a T99 of 8 h against both tested organisms. Furthermore, bactericidal activity was maintained by both antimicrobials for the study duration. Linezolid achieved bactericidal activity, with a T99 of 24 h against both tested isolates. The bactericidal activity of linezolid was maintained for the study duration against the presumptive parent MRSA but not against the VRSA. Vancomycin exhibited no activity against the VRSA isolate. Against the vancomycin-sensitive MRSA isolate, vancomycin exhibited bactericidal activity with a T99 of 24 h. Maximal bacterial reductions of 6, 5.8, and 3.4 log10 CFU/g were noted for daptomycin, quinupristin-dalfopristin, and linezolid, respectively. Daptomycin and quinupristin-dalfopristin demonstrated greater bacterial reductions than that achieved with vancomycin against both isolates at 24, 48, and 72 h (P < 0.05), while linezolid demonstrated greater activity than vancomycin only against the presumptive parent MRSA. There were no significant (>1 dilution) changes in postexposure MICs, and there was no observable resistance to daptomycin, quinupristin-dalfopristin, and linezolid.

The need for optimal agents that exhibit pronounced bactericidal activity in difficult infections that consist of high inocula, protein, and difficult penetration barriers is apparent to promote not only clinical cure but also eradication of resistant organisms. Pharmacodynamic observations in this study for daptomycin and quinupristin-dalfopristin, including rapid and sustained bactericidal activity, are consistent with previous reports of endocarditis simulations against staphylococci (1, 5, 15). Furthermore, the high protein binding affinity of daptomycin does not appear to hamper its activity in simulated endocardial vegetations (1). As expected, vancomycin exhibited no kill activity against the vancomycin-resistant isolate. Overall, linezolid achieved rates and extent of bacterial kill activity against both staphylococcal isolates that were similar to those of vancomycin against the vancomycin-sensitive isolate. These observations with linezolid are consistent with previous staphylococcus-related endocarditis experiments performed with rabbits (11). It appears that development of vancomycin resistance does not hamper the utility of newer antimicrobial options with different mechanisms of action. The utility of novel alternatives and conventional agents that exhibit favorable susceptibilities should be further evaluated against VRSA with larger and longer in vivo endocarditis studies. Now, with the recent identification of S. aureus strains that are resistant to linezolid or quinupristin-dalfopristin, the search for optimal alternatives for vancomycin-resistant staphylococci is imperative (12, 28).

We thank Charles Pelouquin from the Division of Infectious Diseases at the National Jewish Medical and Research Center (Denver, Colo.) for his analysis of linezolid samples. A research grant for this investigation was generously provided by Cubist Pharmaceuticals.

REFERENCES


