Activities of Trovafloxacin and Ampicillin-Sulbactam Alone or in Combination versus Three Strains of Vancomycin-Intermediate Staphylococcus aureus in an In Vitro Pharmacodynamic Infection Model

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The recent isolation of clinical strains of methicillin-resistant Staphylococcus aureus (MRSA) with intermediate susceptibility (MICs, 8 μg/ml) to vancomycin (vancomycin-intermediate S. aureus [VISA]) emphasizes the importance of developing novel antimicrobial regimens and/or agents for future treatment. We studied the activities of ampicillin-sulbactam and trovafloxacin alone or in combination against three unique strains of VISA in an in vitro infection model. Two VISA strains were trovafloxacin susceptible (MICs, ≤2 μg/ml); one VISA strain was trovafloxacin resistant (MIC, 4 μg/ml). Trovafloxacin was administered to simulate a dose of 200 or 400 mg every 24 h. Ampicillin-sulbactam was administered to simulate a dose of 3 g every 6 h. Samples were removed from the infection models over 48 h, and reductions in colony counts were compared between regimens. Trovafloxacin (200 mg) produced rapid killing of a control MRSA strain over the 48-h experiment but produced only slight killing of all three VISA strains. The higher dose of trovafloxacin improved killing but did not produce bactericidal activity at 48 h. Ampicillin-sulbactam produced rapid bactericidal activity against all four strains tested, and colony counts at 8 h were at the limits of detection. However, regrowth occurred by 48 h for each strain. The combination of ampicillin-sulbactam and trovafloxacin provided additive activity against two of the three VISA strains. In conclusion, trovafloxacin or ampicillin-sulbactam alone did not provide adequate activity against the VISA strains for the 48-h evaluation period, but the combination could help improve activity against some strains of VISA.

Infections due to methicillin-resistant Staphylococcus aureus (MRSA) continue to be a significant problem in the 1990s, especially since the glycopeptide antibiotic vancomycin often is the only antimicrobial agent available with reliable activity. The recent isolation of MRSA with intermediate susceptibility (MICs, 8 μg/ml) to vancomycin (vancomycin-intermediate S. aureus [VISA]) in both Japan and the United States (8, 9, 10) indicates that MRSA soon will become fully resistant to the last line of defense against this virulent organism. The expression of decreased vancomycin susceptibility in staphylococci is heterogeneous (1, 18; J. M. Boyce, A. A. Medeiros, and K. Hiramatsu, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. LB-15, 1997; K. Hiramatsu, H. Hanaki, S. Boyle-Vavra, R. S. Daum, H. Labischinski, and F. C. Tenover, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C-166, 1997) and appears to be associated with thickened cell walls (25, 27, 29–31) and increased production of cell wall precursors (29, 30). Interestingly, these strains express increased quantities of penicillin binding proteins (PBPs) (17, 21) and have improved susceptibility to methicillin (30).

The recent isolation of clinical strains of VISA emphasizes the importance of developing novel antimicrobial regimens and/or agents for future treatment considerations. Ampicillin-sulbactam could potentially have activity against VISA isolates that express increased quantities of PBPs. Trovafloxacin is a fluorquinolone that has improved activity against gram-positive organisms. The combination of these two drugs has recently reported to improve activity against some strains of vancomycin-resistant Enterococcus faecium (33). We studied the activities of ampicillin-sulbactam and trovafloxacin alone or in combination against three unique strains of VISA using a one-compartment in vitro pharmacodynamic infection model.

In vitro susceptibility testing, antimicrobial agents, and test media. Ampicillin-sulbactam powder for injection (Unasyn; lot 0088A; Pfizer) and vancomycin (lot 35H040425; Sigma Chemical Company, St. Louis, Mo.) were commercially purchased. Trovafloxacin (Trovan; lot 25381-086-02) was supplied by Pfizer Pharmaceuticals. Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) supplemented with calcium (25 mg/liter) and magnesium (12.5 mg/liter) (SMHB) was used for broth susceptibility testing and in all in vitro infection models. Tryptic soy agar (Mu50) was used for broth susceptibility testing and in vivo infection models. Tryptic soy agar (Mu50) was used for broth susceptibility testing and in vivo infection models. Tryptic soy agar (Mu50) was used for broth susceptibility testing and in vivo infection models. Tryptic soy agar (Mu50) was used for broth susceptibility testing and in vivo infection models. Tryptic soy agar (Mu50) was used for broth susceptibility testing and in vivo infection models.

MATERIALS AND METHODS

Bacterial strains. The three VISA strains tested in this investigation were 14379 (isolated from a dialysis patient with peritonitis; William Beaumont Hospital, Royal Oak, Mich.) (J. Mitchell, M. Ionescu, D. Farnaz, S. Donabedian, M. B. Perri, L. A. Thal, J. Sunstrum, J. W. Chow, T. Smith, and M. J. Zervos, Abstr. 37th Intersci. Conf. Antimicrob. Agents Chemother., abstr. LB-14, 1997), Mu50 (isolated from a pediatric patient with a surgical wound infection; Juntendo Hospital, Tokyo, Japan) (18), and 992 (isolated from a patient in New Jersey with bacteremia; Centers for Disease Control, Atlanta, Ga.) (9). A clinical strain of heterogeneous MRSA (494) was used to compare the activities of the tested antibiotics against VISA strains to that against a vancomycin-sensitive strain.

In vitro susceptibility testing, antimicrobial agents, and test media. Ampicillin-sulbactam powder for injection (Unasyn; lot 0088A; Pfizer) and vancomycin (lot 35H040425; Sigma Chemical Company, St. Louis, Mo.) were commercially purchased. Trovafloxacin (Trovan; lot 25381-086-02) was supplied by Pfizer Pharmaceuticals. Mueller-Hinton broth (Difco Laboratories, Detroit, Mich.) supplemented with calcium (25 mg/liter) and magnesium (12.5 mg/liter) (SMHB) was used for broth susceptibility testing and in all in vitro infection models. Tryptic soy agar (Mu50) was used for broth susceptibility testing and in vivo infection models. Tryptic soy agar (Mu50) was used for broth susceptibility testing and in vivo infection models.
In vitro pharmacodynamic infection model. The in vitro infection model consisted of a 250-ml one-compartment glass chamber with ports for the addition and removal of SMHB, injection of antibiotics, and removal of samples. Prior to each experiment, colonies from overnight growth of bacteria on TSA were added to SMHB as necessary to obtain a 10³ CFU/ml suspension. A 2.5-ml volume of this suspension was added to the infection chamber to produce a starting inoculum of 10⁶ CFU/ml. Fresh stock solutions of trovafloxacin and ampicillin-sulbactam were prepared daily and were stored at 2 to 8°C between dose administration times. Trovafloxacin was administered every 24 h to simulate 100 expected peaks during a 24- or a 400-mg intravenous dose (2.3 or 4.5 g/ml, respectively) (32). Ampicillin-sulbactam was administered to simulate the peak concentrations obtained during a regimen of 3 g every 6 h (approximately 100 and 50 µg/ml, respectively) (14). A dose of 400 mg every 24 h was simulated for trovafloxacin during the combination regimens. Antibiotics were injected into the model over 30 s with a hypodermic syringe. A peristaltic pump (Masterflex; Cole-Parmer Instrument Company, Chicago, Ill.) was used to displace antibiotic-containing medium with fresh SMHB to simulate the half-life of trovafloxacin (12 h) or ampicillin-sulbactam (1 h) (14, 32). A supplemental chamber was used during combination regimens to delay the elimination of the longer-half-life drug (trovafloxacin) from the infection chamber as previously described (5). The glass model apparatus was placed in a water bath and maintained at 37°C for the entire 48-h study period. Each experimental regimen was performed in duplicate in order to ensure reproducibility.

Pharmacokinetic analyses. Samples (0.5 ml) were removed from each infection model at 0, 0.5, 1, 2, 4, 6, 8, 24, 28, 30, 32, and 48 h. Each sample was serially diluted in cold 0.9% sodium chloride, and bacterial counts were determined by placing 20 µl spots of the appropriately diluted samples in triplicate on TSA and incubating them at 37°C for 24 h. We determined these methods to have a limit of detection of 2 log₁₀ CFU/ml (24). Antibiotic carveroy was considered insignificant, since the peak concentrations of antibiotics were in the range of one to four times the MICs for all tested strains. For all samples within 4 h of these peak concentrations, the viable log₁₀ CFU per milliliter was determined from at least the second 10-fold serial dilution—the concentrations in these diluted samples ranged from 1/40 to 1/100 times the MIC. Average colony counts (log₁₀ CFU per milliliter) in the infection models were plotted against time to generate time-kill curves. Duplicate values were averaged. The reductions in the log₁₀ CFU per milliliter over 48 h were determined and compared between regimens. Bactericidal activity was defined as a ≥3-log₁₀ CFU/ml reduction from the starting inoculum. Synergy and additivity between the antimicrobial agents were defined as 0.0 to 0.2-log₁₀ CFU/ml reductions in colony counts at 48 h compared to the results obtained with the most active agent alone. The time to achieve 99.9% killing was determined by linear regression (if R² was ≥0.95) or by visual inspection of the time-kill curves.

TABLE 1. MICs and MBCs for various strains

<table>
<thead>
<tr>
<th>Organism</th>
<th>Vancomycin MIC</th>
<th>Trovafloxacin MBC</th>
<th>Ampicillin-sulbactam MIC</th>
</tr>
</thead>
<tbody>
<tr>
<td>14379</td>
<td>8</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Mu50</td>
<td>8</td>
<td>12</td>
<td>2</td>
</tr>
<tr>
<td>992</td>
<td>6</td>
<td>6</td>
<td>1</td>
</tr>
<tr>
<td>494 (control strain)</td>
<td>0.75</td>
<td>1</td>
<td>0.015</td>
</tr>
</tbody>
</table>

RESULTS

**Susceptibility testing.** The MICs and MBCs for strains 14379, Mu50, 992, and 494 are summarized in Table 1. Strain 992 was the strain most sensitive to ampicillin-sulbactam. For all VISA strains, the trovafloxacin MICs were ≥1 µg/ml, and the trovafloxacin MIC for strain 494 was 0.015 µg/ml.

**In vitro infection models.** (i) Pharmacokinetics. The mean ± standard deviation (SD) peak, trough, half-life, and area under the curve from 0 to 24 h (AUC₀₋₂₄) for trovafloxacin (200 mg every 24 h) were 2.7 ± 0.3 µg/ml, 0.9 ± 0.3 µg/ml, 15.9 ± 4.9 h, and 38.1 ± 5.1 µg·h/ml, respectively. The mean ± SD peak, trough, half-life, and AUC₀₋₂₄ for ampicillin-sulbactam (400 mg every 24 h) were 4.9 ± 0.3 µg/ml, 1.7 ± 0.1 µg/ml, 13.6 ± 0.7 h, and 67.2 ± 5.0 µg·h/ml, respectively. The mean ± SD peak, trough, half-life, and AUC₀₋₂₄ for ampicillin-sulbactam (administered every 6 h) were 103.8 ± 16.3 µg/ml, 2.5 ± 0.4 µg/ml, 0.98 ± 0.11 h, and 575.1 ± 100.2 µg·h/ml, respectively.

(ii) Trovafloxacin regimens. The results for regimens in which trovafloxacin was given every 24 h are shown in Table 2 and Fig. 1. Trovafloxacin produced rapid and complete killing of strain 494 over the 48-h experiment. Trovafloxacin at 200 mg every 24 h produced only slight killing of all three VISA strains, and colony counts at 8 h were significantly higher for strain Mu50 than for all the other strains. Regrowth occurred in all VISA infection models by 24 h, and a blunted effect was observed with the second doses. Trovafloxacin at 400 mg every 24 h caused significantly lower colony counts for all VISA strains at both the 8-h and the 48-h time points.

(iii) Ampicillin-sulbactam regimens. The results for the regimen in which ampicillin-sulbactam was given every 6 h are summarized in Table 3 and Fig. 2. Ampicillin-sulbactam produced rapid bactericidal activity against all four strains tested, and colony counts at 8 h were at or below the limits of detection. This regimen produced significantly lower colony counts for each organism at both the 8-h and the 48-h time points compared with the trovafloxacin (200 mg) regimen. Regrowth started at 24 h and continued at each time point until 48 h for every strain except 992. The colony counts of strain 992 were statistically lower between 24 and 48 h than were those of all other organisms.

(iv) Ampicillin-sulbactam–trovafloxacin combination regimens. The results obtained for the combination models are summarized in Table 3 and Fig. 3. The combination of ampicillin-sulbactam and trovafloxacin provided additional 2.7- and 0.7-log₁₀ CFU/ml reductions in colony counts for strains 14379 and 992 but provided no additional activity compared to the

**TABLE 2. Colony counts at 8 and 48 h in the trovafloxacin regimens**

<table>
<thead>
<tr>
<th>Organism</th>
<th>200 mg every 24 h</th>
<th>400 mg every 24 h</th>
</tr>
</thead>
<tbody>
<tr>
<td>8 h</td>
<td>48 h</td>
<td>8 h</td>
</tr>
<tr>
<td>14379</td>
<td>3.6 ± 0.1</td>
<td>7.9 ± 0.1</td>
</tr>
<tr>
<td>992</td>
<td>3.4 ± 0.4</td>
<td>7.6 ± 0.8</td>
</tr>
<tr>
<td>Mu50</td>
<td>5.6 ± 0.0</td>
<td>8.4 ± 0.0</td>
</tr>
<tr>
<td>494 (control strain)</td>
<td>4.7 ± 0.4</td>
<td>2.0 ± 0.0</td>
</tr>
</tbody>
</table>

*For all organisms, colony counts were significantly lower at 8 h in the higher-dose trovafloxacin regimen. Colony counts at 48 h for strains 14379 and 992 were significantly lower in the higher-dose trovafloxacin regimen. Colony counts were below the limits of detection. Significantly lower than values for other strains during the low- or high-dose simulation.
FIG. 1. Activities of trovafloxacin at 200 mg (A) or 400 mg (B) administered every 24 h against VISA strains in the in vitro infection model. Growth controls are represented by the corresponding filled symbols for each strain. Error bars represent SDs.
most active monotherapy regimen for strain Mu50 and the control strain (strain 494).

**DISCUSSION**

The recent reports of MRSA with decreased vancomycin susceptibility are a grim indication that therapeutic failures against these pathogens soon will be related to vancomycin resistance (8–10, 17, 18, 21). The mechanisms that produce and regulate this decreased vancomycin susceptibility are incompletely determined, but the presence of the enterococcal van gene family has not been detected in VISA (Hiramatsu et al., 37th ICAAC; Mitchell et al., 37th ICAAC; R. F. Pfeltz, M. A. Batten, C. Baranyk, R. K. Jayaswal, and B. J. Wilkinson, Abstr. 98th Gen. Meet. Am. Soc. Microbiol., abstr. A-19, 1998). This glycopeptide resistance results in decreased susceptibility to other glycopeptide antimicrobial agents, such as teicoplanin and LY333328 (1, 3, 27, 29, 30; J. R. Aeschlimann, E. Hershberger, and M. J. Rybak, submitted for publication), so alternate classes of antimicrobial agents will be necessary to treat VISA infections.

Trovafloxacin is a fluoroquinolone antimicrobial agent which has greatly improved activity against gram-positive pathogens (11, 28) and might have adequate activity against a VISA isolate. As a threefold increase in the expression of PBPs 2 and 2’ has been reported for Mu50 (17) as well as for in vitro vancomycin-resistant staphylococcal mutants (21), we hypothesized that the administration of higher doses of ampicillin-sulbactam might also provide adequate anti-VISA activity, since ampicillin has a good affinity for these PBPs (2).

The activity that we observed in the infection models for trovafloxacin against VISA was predictably weaker than the activity against strain 494, based on the lower MIC for this strain, the known concentration-dependent killing activity of fluoroquinolones, and the pharmacodynamic predictors of activities for these agents (19). Although currently somewhat controversial, an AUC₀–2₄/MIC ratio of >100 appears to be necessary for adequate fluoroquinolone activity against gram-positive bacteria. This ratio was met only against the MRSA

<table>
<thead>
<tr>
<th>Organism</th>
<th>Colony counts (log₁₀ CFU/ml ± SD) at the indicated time for ampicillin-sulbactam at 3 g every 6 h</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Without trovafloxacin</td>
</tr>
<tr>
<td></td>
<td>8 h</td>
</tr>
<tr>
<td>14379</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>992</td>
<td>2.0 ± 0.0°</td>
</tr>
<tr>
<td>Mu50</td>
<td>2.1 ± 0.1</td>
</tr>
<tr>
<td>494 (control strain)</td>
<td>2.0 ± 0.0°</td>
</tr>
</tbody>
</table>

° Colony counts were below the limits of detection.

* Significantly lower than values for all other strains during ampicillin-sulbactam monotherapy.

![FIG. 2. Activity of ampicillin-sulbactam administered every 6 h against VISA strains in the in vitro infection model. Growth controls are represented by the corresponding filled symbols for each strain. Error bars represent SDs.](http://aac.asm.org/ on June 24, 2017 by guest)
control strain (strain 494). Higher doses of trovafloxacin did significantly increase VISA killing but still did not provide bactericidal activity (as measured at 48 h). Activity against all strains (quantified by the viable log_{10} CFU per milliliter at 48 h) was significantly correlated ($R^2$, 0.76; $P$, <0.05) with the log AUC_{0–24}/MIC ratio—a finding which agrees with the findings of previous studies of fluoroquinolone activity against _S. aureus_ (13).

Ampicillin-sulbactam activity in the infection models was highest against strain 992. The killing activity of the first ampicillin-sulbactam dose was rapid against all VISA strains, and each was killed to the limit of detection by 8 h, but regrowth did occur between 24 and 48 h for all of the strains. It is unclear if the regrowth that we observed in the infection models was due to the emergence of resistance or if it simply reflected suboptimal antibiotic exposures. As noted, we did not observe changes in MICs. We and others previously have observed this regrowth phenomenon (7, 22). Regrowth in the in vitro infection models appears to occur most often for antibiotics with time-dependent activity during pharmacokinetic simulations, where concentrations either stay just above the MIC or fall below the MIC during the course of dosing intervals. Indeed, regrowth in our ampicillin-sulbactam infection models appeared to be inversely related to the time (from 0 to 24 h) that concentrations were above the MICs for the organisms (approximately 15, 11, 7, and 3 h for strains 992, 494, 14379, and Mu50, respectively). Administration of ampicillin-sulbactam every 4 h or as a continuous infusion could be a way to improve the time above the MIC to decrease the degree of regrowth. This regrowth may be of questionable significance, since the rapid initial clearance of the bacteria coupled with a functioning immune system could be adequate enough to cure a VISA infection. The successful treatment of such an infection with ampicillin-sulbactam plus arbekacin (10) provides encouraging support for this hypothesis.

In conclusion, we determined that trovafloxacin administered at 200 mg every 24 h likely will not provide adequate activity against the VISA strains isolated thus far. Trovafloxacin administered at 400 mg every 24 h could improve activity. Ampicillin-sulbactam had good initial activity against all three VISA strains, followed by bacterial regrowth. The combination of these antimicrobial agents resulted in some additive activity against two of the VISA strains and could represent a viable treatment strategy. Although the therapeutic use of trovafloxacin is now limited because of hepatotoxicity, the Food and Drug Administration has advised physicians that its use should be restricted to short-term treatment (<14 days) of “serious, life- or limb-threatening infections...[when] the treating physician believes that...the benefit of the product for the patient outweighs the potential risk” (M. M. Lumpkin, Trovan health advisory; http://www.fda.gov/cder/news/trovan/trovan-advisory.htm). Clearly, an infection with VISA could fit these criteria.

**ACKNOWLEDGMENTS**

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