Serum Bactericidal Activity of the Methoxyfluoroquinolones Gatifloxacin and Moxifloxacin against Clinical Isolates of Staphylococcus Species: Are the Susceptibility Breakpoints Too High?

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Healthy volunteers received a single dose of gatifloxacin and moxifloxacin (400 mg each), and serum samples were obtained from these volunteers over a 24-h period. Prolonged (≥12 h) serum bactericidal activity (SBA) was observed for both agents against staphylococcal isolates with minimum inhibitory concentrations (MICs) of gatifloxacin of =≤0.5 μg/mL. In strains with gatifloxacin MICs of 1.0 μg/mL, SBA was observed for ≈6 h, and, for isolates with gatifloxacin MICs of 2.0 μg/mL, little or no SBA was observed for either drug. The relative lack of SBA against less susceptible strains of staphylococci suggests that the current susceptibility breakpoint concentration (MIC, 2.0 μg/mL) for these methoxyfluoroquinolones against Staphylococcus is too high.

The methoxyfluoroquinolones gatifloxacin and moxifloxacin have exceptional in vitro activity against staphylococci, including many methicillin-resistant strains [1,2]. The susceptibility breakpoint concentration established by the US Food and Drug Administration (FDA) for gatifloxacin and moxifloxacin and by NCCLS for gatifloxacin is 2.0 μg/mL for Staphylococcus species. Virtually all methicillin-sensitive Staphylococcus aureus (MSSA) isolates have MICs well below this susceptibility breakpoint, but many methicillin-resistant S. aureus (MRSA) strains have MICs that border this concentration [1, 2]. This is especially true for moxifloxacin (MIC90 of ≈4.0 μg/mL).

In vitro pharmacodynamic studies and animal infection models that have correlated fluoroquinolone concentrations with resultant killing of S. aureus do not support the current susceptibility breakpoint concentration [3]. Because there is a paucity of clinical data evaluating these methoxyfluoroquinolones against less susceptible strains of staphylococci, these pharmacodynamic studies have not been confirmed or refuted. In this investigation, we measured the serum bactericidal activity over time of gatifloxacin and moxifloxacin against a range of well-defined clinical strains of MSSA and MRSA. By analyzing less-susceptible strains, we aspired to further define an appropriate susceptibility breakpoint concentration for these methoxyfluoroquinolones against Staphylococcus species.

Subjects and methods. Appropriate informed consent was obtained from the research subjects in this study, and all guidelines for human experimentation were followed in the conduct of this research study. Eleven healthy male volunteers participated in this investigation. Each gave written informed consent that was approved by the University Committee on Research in Human Subjects at Michigan State University. None of these subjects had a history of chronic disease or were receiving medications. These volunteers had a mean age of 36 years (range, 27–54 years) and mean weight of 86 kg (range, 62–114 kg). All were within 20% of their ideal body weight.

Each subject received a single oral dose of gatifloxacin (Bristol-Myers Squibb) and moxifloxacin (Bayer) (400 mg each). Study volunteers initially received gatifloxacin, and, after a 1-week washout period, each subject received a dose of moxifloxacin. The study antibiotics were taken on an empty stomach following a 12 h fast. Food intake was allowed 2 h after receiving the dose of antibiotic.

Venous blood samples were obtained immediately before each drug was administered (at time 0), to use as controls, and at 2, 6, 12, and 24 h after the dose of each drug was administered. Following centrifugation, serum samples were aliquotted and stored at −70°C until the time of analysis. Serum concentrations of gatifloxacin and moxifloxacin were measured at each time period by a validated high performance liquid chromatographic method [4].

The study isolates were obtained from clinical microbiology laboratories and included 3 strains each of MSSA and MRSA, plus 1 methicillin-sensitive and 1 methicillin-resistant isolate of a coagulase-negative staphylococcus. Specific strains were chosen in order to study a range of susceptible isolates (with...
MICs of 0.06–2.0 μg/mL. The inhibitory activities of gatifloxacin and moxifloxacin were determined by microdilution methodology, as recommended by the NCCLS. The MIC was defined as the lowest concentration of antibiotic that prevented visible growth. PCR products encompassing the quinolone resistance-determining regions (QRDRs) of grlA and gyrA of each strain were obtained using primers and parameters described elsewhere [5]. Nucleotide sequencing was performed at the Center for Molecular Medicine and Genetics Macromolecular Core Facility at Wayne State University (Detroit, MI) by the dideoxy chain-termination method using the Applied Biosystems (ABI) 377 automated capillary-based system (Perkin-Elmer Applied Biosystems).

Serum inhibitory titers and bactericidal titers were determined according to NCCLS standards. Each determination was performed in duplicate. Wells with no visible growth and the first well that showed growth were subcultured onto supplemented Mueller-Hinton agar, and plates were incubated for 2 days prior to counting colonies. Each isolate was tested against serum samples obtained at each time point for all subjects. The serum bacterial titer end point was defined as the highest dilution of serum yielding a 99.9% kill rate. The median and geometric mean serum inhibitory titer and bacterial titer at each time point was calculated and plotted.

Results. Each of the 11 subjects received the study fluoroquinolones according to protocol, and no adverse experiences were observed or reported. The MICs of the study strains and mutations resulting in amino acid substitutions in the QRDR regions are presented in Table 1. The in vitro potency of the 2 methoxyfluoroquinolones was found to be similar for both the methicillin-sensitive and methicillin-resistant strains. All of these isolates would be considered susceptible (MICs of ≤0.2 μg/mL) to gatifloxacin and moxifloxacin based on the current susceptibility breakpoint. Double QRDR mutations (i.e., mutations in both grlA and gyrA) were found in the less susceptible strains (those with gatifloxacin MICs of ≥1.0 μg/mL).

The mean serum concentrations of gatifloxacin and moxifloxacin in these subjects have been previously published [6]. Both agents exhibited rapid serum inhibitory and bactericidal activity (for 2 h) and prolonged activity (for ≥12 h) against methicillin-sensitive and methicillin-resistant strains devoid of QRDR mutations (those with gatifloxacin MICs of ≤0.5 μg/mL). Against isolates with dual mutations, the duration of the serum bactericidal activity was decreased to ≤6 h. Little to no inhibitory or bactericidal activity was observed in isolates with gatifloxacin MICs of 2.0 μg/mL for either methoxyfluoroquinolone (figure 1).

Discussion. Experimental models of infection and clinical studies have shown that bactericidal activity predicts therapeutic efficacy and results in improved clinical outcome. Bactericidal activity is especially important for infections in which antimicrobial penetration and host defense mechanisms at the site of infection are limited. Staphylococci are a major cause of these types of infections, which include endocarditis, osteomyelitis, and bacteremia in the neutropenic host. The need for prolonged bactericidal activity has been observed in vitro and in animal infection models with various fluoroquinolones [7, 8]. The lack of prolonged inhibitory and bactericidal activity against S. aureus isolates with gatifloxacin MICs of ≥1.0 μg/mL in this study is of concern because these strains would be considered susceptible isolates based on the current susceptibility breakpoint concentration. Even at steady-state, the concentrations of these methoxyfluoroquinolones would not increase appreciably enough to significantly prolong the duration of their bactericidal activity.

The findings from this study were not totally unexpected, given the findings of previous investigations. In an in vitro dynamic model, MIC breakpoints of 0.4 μg/mL for moxifloxacin and 0.3 μg/mL for gatifloxacin were determined based on their bactericidal and regrowth kinetics [9, 10]. At these MIC breakpoint concentrations, the ratios of AUC24 to MIC for either methoxyfluoroquinolone would be in the range of 80–100. In murine infection models, Andes and Craig [11] found that a mean ratio of AUC24 to MIC of 77 was required for 1 log10 killing of S. aureus by gatifloxacin. In time-kill studies, Lister [12] observed rapid regrowth of staphylococci with moxifloxacin MICs of ≥0.5 μg/mL following exposure to peak serum levels, achieved with a 400-mg dose. In a similar model, a 4-fold increase in the MIC of a less susceptible strain of MRSA (MIC, 2 μg/mL) was observed following exposure to moxifloxacin [13]. All of these pharmacodynamic studies suggest that the likelihood of treatment failure increases when using these fluoroquinolones against staphylococcal isolates with reduced susceptibility.

### Table 1. In vitro activity of gatifloxacin and moxifloxacin and sequencing results obtained for methicillin-sensitive and methicillin-resistant Staphylococcus species study isolates.

<table>
<thead>
<tr>
<th>Staphylococcus strains</th>
<th>MICs, μg/mL</th>
<th>Mutations</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gat</td>
<td>Mox</td>
</tr>
<tr>
<td>Methicillin-sensitive</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolate 1, S. aureus</td>
<td>0.125</td>
<td>0.06</td>
</tr>
<tr>
<td>Isolate 2, CNS</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Isolate 3, S. aureus</td>
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<td>0.5</td>
</tr>
<tr>
<td>Isolate 4, S. aureus</td>
<td>2.0</td>
<td>1.0</td>
</tr>
<tr>
<td>Methicillin-resistant</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Isolate 5, S. aureus</td>
<td>0.06</td>
<td>0.06</td>
</tr>
<tr>
<td>Isolate 6, CNS</td>
<td>0.5</td>
<td>0.25</td>
</tr>
<tr>
<td>Isolate 7, S. aureus</td>
<td>1.0</td>
<td>0.5</td>
</tr>
<tr>
<td>Isolate 8, S. aureus</td>
<td>2.0</td>
<td>1.0</td>
</tr>
</tbody>
</table>

NOTE. CNS, coagulase-negative Staphylococcus; Gat, gatifloxacin; Mox, moxifloxacin.
Figure 1. Median serum bactericidal titers (SBT), over time (hours after administration), of gatifloxacin (◇) and moxifloxacin (▲) for treatment of staphylococcal strains. CNS, coagulase-negative staphylococcus; MRSA, methicillin-resistant Staphylococcus aureus; MSSA, methicillin-sensitive S. aureus.

In this study, both gatifloxacin and moxifloxacin exhibited prolonged bactericidal activity against coagulase-positive and coagulase-negative staphylococci as well as methicillin-sensitive and methicillin-resistant strains. The MIC of a given isolate appears to be the most important parameter in determining the inhibitory and bactericidal activity of these methoxyfluoroquinolones. A lowering of the susceptibility breakpoint concentration for gatifloxacin and moxifloxacin against Staphylo-
coccus species is suggested, given the findings of this investigation and other pharmacodynamic studies. Treating infections with staphylococcus strains that have MICs ≤0.5 μg/mL allows for prolonged inhibitory and bactericidal activity in serum. By using this lowered breakpoint concentration for susceptible strains, clinicians may improve clinical outcomes by decreasing the likelihood of bacterial regrowth and the development of resistance [8, 14]. This could be especially important when treating serious staphylococcal infections, such as osteomyelitis.

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References