Synergy between gemifloxacin and trimethoprim/sulfamethoxazole against community-associated methicillin-resistant Staphylococcus aureus

Steven N. Leonard1,2, Glenn W. Kaatz1,3,4, Latoyia R. Rucker1 and Michael J. Rybak1–3*

1Anti-Infective Research Laboratory, Eugene Applebaum College of Pharmacy and Health Sciences, Wayne State University, 259 Mack Avenue, Detroit, MI 48201, USA; 2Detroit Receiving Hospital, 4201 Saint Antoine Street, Detroit, MI 48201, USA; 3School of Medicine, Wayne State University, Detroit, MI 48201, USA; 4John D. Dingell V A Medical Center, 4646 John R Street, Detroit, MI 48201, USA

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Objectives: The rapid emergence of methicillin-resistant Staphylococcus aureus from the community (CA-MRSA) presents difficulties in making treatment choices. We evaluated whether combining another orally available agent commonly used to treat CA-MRSA with gemifloxacin would enhance gemifloxacin activity against CA-MRSA.

Methods: Fifty strains of SCCmec IV, agr group 1, Panton–Valentine leucocidin-positive CA-MRSA were evaluated for susceptibilities to gemifloxacin, trimethoprim/sulfamethoxazole, doxycycline, levofloxacin, rifampicin, clindamycin and erythromycin. Twenty of these strains were evaluated for the potential for synergy between gemifloxacin and trimethoprim/sulfamethoxazole, clindamycin and rifampicin by time–kill analysis. Two strains were further evaluated in an in vitro pharmacokinetic/pharmacodynamic (PK/PD) model.

Results: In time–kill analyses, gemifloxacin combined with trimethoprim/sulfamethoxazole produced additivity (6/20) or synergy (11/20) in 85% of the isolates tested. The addition of clindamycin to gemifloxacin showed additivity (3/20) or synergy (2/20) in 25% of the isolates. All isolates displayed indifference to the combination of gemifloxacin and rifampicin. In the PK/PD model, combining gemifloxacin and trimethoprim/sulfamethoxazole provided potent and sustained bactericidal activity to detection limits of 2 log10 cfu/mL by 48 h; gemifloxacin combined with clindamycin or with rifampicin killed to detection limits by 56 h or later. One isolate developed efflux-mediated resistance to gemifloxacin at 96 h with gemifloxacin monotherapy. All combinations prevented the emergence of this resistance.

Conclusions: Synergy or additivity was demonstrated by time–kill analysis between gemifloxacin and trimethoprim/sulfamethoxazole in most isolates tested. In the PK/PD model, the addition of trimethoprim/sulfamethoxazole, clindamycin and rifampicin enhanced the activity of gemifloxacin against CA-MRSA and suppressed the emergence of resistance to gemifloxacin.

Keywords: clindamycin, rifampicin, MRSA

Introduction

The rise of drug-resistant pathogens continues to be a problem worldwide. Methicillin-resistant Staphylococcus aureus (MRSA) is of particular concern. Historically, MRSA was largely confined to healthcare institutions (HA-MRSA); however, it is increasingly causing infections in the community (CA-MRSA).1,2 CA-MRSA is distinctly different from HA-MRSA in that it possesses a smaller staphylococcal cassette chromosome (SCCmec IV) and is more susceptible to non-β-lactam antibiotics.3,4 Fluoroquinolones have been investigated for a variety of infections caused by S. aureus.3,6 They possess concentration-dependent bactericidal activity and rapidly penetrate to sites of infection. However, the rapid emergence of resistance to older generations of these
compounds has raised concerns. Newer-generation fluoroquinolones have improved activity at topoisomerase IV, which is the primary target site in *S. aureus*. However, there are still concerns for resistance; therefore, these drugs are not recommended for the treatment of MRSA infections.

Gemifloxacin is a broad-spectrum fluoroquinolone that is approved by the FDA for the treatment of community-acquired pneumonia and acute exacerbation of chronic bronchitis. It has been reported that it has improved activity against Gram-positive pathogens compared with the older-generation fluoroquinolones. Like other quinolones, gemifloxacin is less potent against MRSA than it is against other Gram-positive cocci such as *Streptococcus pneumoniae*. However, it has improved activity over the older-generation fluoroquinolones and may retain activity against some ciprofloxacin-resistant strains of *S. aureus*. Although not recommended as a primary treatment option for MRSA, the combination of newer fluoroquinolones with other active anti-MRSA agents may improve therapy and decrease the potential for the emergence of resistance. In the current investigation, we evaluated the potential for synergy between gemifloxacin and trimethoprim/sulfamethoxazole, clindamycin and rifampicin by time–kill analysis, and the activity of these agents alone and in combination in an in vitro pharmacokinetic/pharmacodynamic (PK/PD) model.

Materials and methods

Bacterial strains

Fifty clinical non-duplicate isolates of SCCmeC IV, accessory gene regulator (agr) group I, Panton–Valentine leucocidin (PVL)-positive CA-MRSA isolated from patients at the Detroit Medical Center were evaluated for susceptibility. Seventy percent of these isolates were from a skin source. Twenty randomly chosen strains were run in time–kill experiments. Two isolates from those displaying synergy between trimethoprim/sulfamethoxazole and gemifloxacin by time–kill analysis were randomly chosen and evaluated in an in vitro PK/PD model.

Antimicrobials

Gemifloxacin analytical powder was provided by the manufacturer (Oscent Pharmaceuticals, Waltham, MA, USA). Rifampicin, clindamycin, trimethoprim, sulfamethoxazole, doxycycline, levofloxacin and erythromycin were commercially purchased (Sigma Chemical Company, St Louis, MO, USA).

Media

Mueller–Hinton broth (Difco, Detroit, MI, USA) supplemented with 25 mg/L calcium and 12.5 mg/L magnesium (SMHB) was used for all susceptibility testing, time–kill experiments and in vitro PK/PD models. Colony counts were determined using tryptic soy agar (TSA) (Difco) plates. Etests were performed on Mueller–Hinton agar (MHA) (Difco) plates.

Susceptibility testing

MICs of study antimicrobial agents were determined by broth microdilution in SMHB according to CLSI guidelines. Changes in susceptibility from the PK/PD model were evaluated by Etest. The presence of inducible clindamycin resistance was determined by the disc diffusion test (D-test).

Molecular characterization

The SCCmeC type and the presence of the genes encoding PVL were determined by multiplex PCR by a previously described method. Characterization of agr type was performed using multiplex PCR and agr group-specific primers.

Synergy testing

Potential for synergy was evaluated employing 20 CA-MRSA strains by time–kill methods in duplicate at an inoculum of ~10^6 cfu/mL. Time–kill experiments were initially performed at both 1 × and 0.5 × MIC. Similar results were observed and 0.5 × MIC was chosen for the duration of the experiments. All results presented are from time–kill experiments performed at 0.5 × MIC. Aliquots (0.1 mL) were removed at 0, 1, 4, 8 and 24 h and serially diluted in cold 0.9% sodium chloride. Bacterial counts were determined by plating appropriate dilutions using an automatic spiral plater (WASP, DW Scientific, West Yorkshire, UK) and counting colonies using the ProtoCol colony counter (Synoptics Limited, Frederick, MD, USA). The lower limit of reliable detection for colony counts was 2 log_{10} cfu/mL. Time–kill curves were constructed by plotting mean colony counts (log_{10} cfu/mL) versus time. Synergy was defined as ≥2 log_{10} cfu/mL increase in killing at 24 h with the combination, in comparison with the killing by the most active single drug. Additivity was defined as a 1–2 log_{10} cfu/mL increase in kill with the combination in comparison with the most active single agent. Indifference was defined as ±1 log_{10} cfu/mL killing or growth. Combinations that resulted in ≥1 log_{10} bacterial growth in comparison with the least active single agent were considered to represent antagonism.

In vitro PK/PD infection model

Two strains were randomly chosen to be run in an in vitro PK/PD model consisting of a 250 mL one-compartment glass apparatus with ports for the addition and removal of media, antibiotics and samples. The model was placed in a water bath at 37°C throughout the simulation with a magnetic stir bar for mixing. Fresh media was continuously supplied and removed via a peristaltic pump (Masterflex, Cole-Parmer Instrument Company, Chicago, IL, USA) set to simulate the half-lives of the antibiotics. A starting inoculum of ~10^7 cfu/mL was used for all simulations. Free drug concentrations were used to simulate regimens of gemifloxacin 320 mg every 24 h (targets: C_{max} 0.5 mg/L, t_{1/2} 7 h), trimethoprim/sulfamethoxazole 160/800 mg every 12 h (targets: C_{max} 1.3/30 mg/L, t_{1/2} 10/10 h, respectively), clindamycin 300 mg every 8 h (targets: C_{max} 1 mg/L, t_{1/2} 3 h) and rifampicin 300 mg every 12 h (targets: C_{max} 0.8 mg/L, t_{1/2} 3 h). All models were done in duplicate.

Pharmacodynamic analysis

Bacterial quantification was determined over 96 h by plating appropriate dilutions onto TSA using the automatic spiral plater and colonies were counted with the ProtoCol counter (lower detection limit 2 log_{10} cfu/mL). Antibiotic carryover for gemifloxacin was minimized by treating samples with non-ionic polymeric adsorbent beads (Amberlite, Sigma Chemical Company) and for all other drugs by serial dilution. The total reduction in log_{10} cfu/mL was determined by plotting time–kill curves of the number of remaining organisms over 96 h. Bactericidal activity was defined as ≥3 log_{10} cfu/mL reduction in colony count from initial inoculum.
Synergy between gemifloxacin and trimethoprim/sulfamethoxazole against CA-MRSA

Table 1. Susceptibility results for 50 CA-MRSA

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>MIC&lt;sub&gt;50&lt;/sub&gt;</th>
<th>MIC&lt;sub&gt;90&lt;/sub&gt;</th>
<th>Range</th>
<th>R2907</th>
<th>R3080</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gemifloxacin</td>
<td>0.03</td>
<td>0.5</td>
<td>0.008–32</td>
<td>0.0625</td>
<td>0.0625</td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0.25</td>
<td>1</td>
<td>0.0625–8</td>
<td>0.5</td>
<td>1</td>
</tr>
<tr>
<td>Trimethoprim/sulfamethoxazole</td>
<td>0.125</td>
<td>0.25</td>
<td>0.015–32</td>
<td>0.125</td>
<td>0.25</td>
</tr>
<tr>
<td>Levofloxacin</td>
<td>0.25</td>
<td>4</td>
<td>0.0625–32</td>
<td>0.25</td>
<td>0.25</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>0.008</td>
<td>0.008</td>
<td>0.004–0.008</td>
<td>0.008</td>
<td>0.008</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>0.25</td>
<td>0.5</td>
<td>0.03–64</td>
<td>0.125</td>
<td>0.125</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>64</td>
<td>128</td>
<td>0.25–256</td>
<td>2</td>
<td>256</td>
</tr>
</tbody>
</table>

MIC<sub>50</sub>, concentration at which 50% of the isolates are inhibited; MIC<sub>90</sub>, concentration at which 90% of the isolates are inhibited. Data are presented in mg/L.

Figure 1. Time–kill curve analysis of one CA-MRSA isolate. All antimicrobials are at a concentration of 0.5 × MIC. This isolate shows synergy between gemifloxacin and clindamycin or rifampicin. Filled circles, growth control (GC); open circles, gemifloxacin; filled triangles, clindamycin; open triangles, rifampicin; filled squares, trimethoprim/sulfamethoxazole; open squares, gemifloxacin + clindamycin; filled diamonds, gemifloxacin + rifampicin; open diamonds, gemifloxacin + trimethoprim/sulfamethoxazole.

Pharmacokinetic analysis

Pharmacokinetic samples were obtained through the injection port over 96 h for verification of antibiotic concentrations. Gemifloxacin and trimethoprim/sulfamethoxazole concentrations were determined by HPLC at Sunnybrook Health Sciences Centre, Toronto, Ontario, Canada, by previously described methods. The lower limit of quantification for gemifloxacin was 15 ng/mL with a CV% of <5%, trimethoprim and sulfamethoxazole both had a lower limit of quantification of 100 ng/mL with a CV% of <5%. Concentrations of rifampicin and clindamycin were determined by bioassay using Micrococcus luteus ATCC 9341. The half-life, area under the curve and peak concentrations were determined by the trapezoidal method utilizing PK Analyst software (version 1.10, MicroMath Scientific Software, Salt Lake City, UT, USA).

Resistance

To screen for the emergence of resistance, 50 μL from the 24, 48, 72 and 96 h samples were plated onto MHA containing 3 × the MIC of the antibiotic. Plates were incubated at 35°C for 48 h and inspected for growth. The MIC of detectable growth on the screening plates was determined by Etest. The possible contribution of efflux to MIC increases in resistant isolates was assessed by determining the microdilution MIC of the common efflux pump substrates acriflavine (ACR), benzalkonium chloride (BAC), ethidium bromide (EtBr) and tetraphenylphosphonium (TPP), as well as gemifloxacin, in the presence and absence of 20 mg/L reserpine. MIC reductions of ≥4-fold in the presence of reserpine were considered indicative of the efflux.

Statistical analysis

Changes in cfu/mL over 0–96 h were evaluated by two-way analysis of variance (ANOVA) with Tukey’s post hoc test. A P value of ≤0.05 was considered significant. All statistical analysis was performed using SPSS Statistical Software (Release 15.0, SPSS, Inc., Chicago, IL, USA).

Table 2. Pharmacokinetics of gemifloxacin, trimethoprim/sulfamethoxazole, clindamycin and rifampicin obtained in the model

<table>
<thead>
<tr>
<th>Antibiotic regimen</th>
<th>fC&lt;sub&gt;max&lt;/sub&gt; (mg/L)</th>
<th>t&lt;sub&gt;1/2&lt;/sub&gt; (h)</th>
<th>fAUC&lt;sub&gt;0–24&lt;/sub&gt; (mg/L·h&lt;sup&gt;−1&lt;/sup&gt;)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gemifloxacin 320 mg every 24 h</td>
<td>0.54 ± 0.08</td>
<td>6.93 ± 2.3</td>
<td>5.39 ± 0.07</td>
</tr>
<tr>
<td>Trimethoprim 160 mg every 12 h</td>
<td>1.51 ± 0.16</td>
<td>10.04 ± 2.91</td>
<td>26.49 ± 0.88</td>
</tr>
<tr>
<td>Sulfamethoxazole 800 mg every 12 h</td>
<td>36.68 ± 0.17</td>
<td>10.18 ± 0.08</td>
<td>683.81 ± 0.26</td>
</tr>
<tr>
<td>Rifampicin 300 mg every 12 h</td>
<td>0.88 ± 0.07</td>
<td>3.09 ± 0.8</td>
<td>14.11 ± 0.2</td>
</tr>
<tr>
<td>Clindamycin 300 mg every 8 h</td>
<td>1.09 ± 0.06</td>
<td>3.35 ± 1</td>
<td>18.37 ± 0.9</td>
</tr>
</tbody>
</table>

fC<sub>max</sub>, free drug peak concentration; fAUC<sub>0–24</sub>, free area under the concentration curve over 24 h. Data are presented as means ± SD.
Results

Susceptibility results for the 50 strains are displayed in Table 1. The MIC$_{50}$ and MIC$_{90}$ of gemifloxacin were 0.03 and 0.5 mg/L, respectively. All strains were SCC$_{mec}$ type IV, agr group I and PVL-positive. Three of 20 strains used in time–kills displayed the inducible macrolide-lincosamide-streptogramin B (iMLS$_B$) phenotype. In time–kill analyses, the addition of trimethoprim/sulfamethoxazole to gemifloxacin showed additivity (6/20) or synergy (11/20) in 85% of isolates tested. The addition of clindamycin to gemifloxacin showed additivity (3/20) or synergy (2/20) in 25% of isolates tested; all three of the isolates displaying iMLS$_B$ showed indifference. All isolates showed indifference to the addition of rifampicin to gemifloxacin. Figure 1 shows an example time–kill curve.

Two strains (R2907 and R3080) were run in the in vitro PK/PD model. Both of these strains displayed synergy between gemifloxacin and trimethoprim/sulfamethoxazole, and indifference to the other combinations in time–kill analyses. Susceptibility results for these two isolates are given in Table 1 and pharmacokinetic results obtained in the model in Table 2. Significantly enhanced killing was observed for all combinations whereas all individual agents displayed regrowth over 96 h ($P < 0.001$). Kill to detection limits (2 log$_{10}$ cfu/mL) occurred at 48 h with gemifloxacin plus trimethoprim/sulfamethoxazole, and at 56 h or later with the other combinations (Figure 2). Against R2907, gemifloxacin alone resulted in an 8-fold increase in MIC to 0.5 mg/L at 96 h; the derived strain was positive for efflux activity. Rifampicin alone resulted in the emergence of resistance (MIC $\geq$ 32 mg/L) at 24 h for both isolates. No other changes in susceptibility were detected. All combinations suppressed the emergence of resistance to gemifloxacin over 96 h.

Discussion

CA-MRSA is a growing threat and has been identified as the cause of infection in otherwise healthy individuals with minimal risk factors for MRSA. Traditionally, the drug of choice for treatment of infections caused by MRSA was vancomycin; however, the emergence of MRSA outside of healthcare institutions has led to an increased need for orally available treatment options. Trimethoprim/sulfamethoxazole has been widely used for the treatment of MRSA infections in the outpatient setting though there is little data to support its use and it has been associated with failures. Fluoroquinolones have shown excellent activity against *S. aureus* in the past; however, rapid emergence of resistance to older generations of these compounds raised concerns over the utility of these agents in the treatment of infections caused by *S. aureus* and they were largely set aside. CA-MRSA, however, are more susceptible to fluoroquinolones compared with HA-MRSA (64% to 88% versus 5% to 12% susceptible, respectively).

In the current investigation, we evaluated the potential for synergy between gemifloxacin and other oral agents active against CA-MRSA. We found additivity or synergy between gemifloxacin and trimethoprim/sulfamethoxazole in 85% of the isolates tested, in 25% for the gemifloxacin and clindamycin combination and all indifferences between gemifloxacin and rifampicin. Rifampicin has previously been studied in combination with the older generation of fluoroquinolones with mixed results reported. A study examining the combination of moxifloxacin and various oral agents with activity against MRSA by chequerboard methodology found mostly additive or indifferent effects when combined with rifampicin, clindamycin and trimethoprim/sulfamethoxazole. None of these studies was specific for CA-MRSA.

In the PK/PD model, gemifloxacin combined with trimethoprim/sulfamethoxazole provided rapid and sustained bactericidal activity to detection limits by 48 h. Gemifloxacin combined with clindamycin and with rifampicin also displayed significantly enhanced killing in the PK/PD model. Improved results over those obtained in the time–kill experiments are not unexpected as there are both therapeutic levels as well as multiple doses given in the model. This is in contrast to the activity of all individual agents alone which all displayed regrowth over 96 h. In one of the two isolates, gemifloxacin alone resulted in the emergence of efflux-mediated resistance at 96 h. All combinations with gemifloxacin suppressed the emergence of this resistance. Although 20 strains were tested for the potential for synergy, caution is warranted when interpreting and generalizing these
results as PK/PD model data were generated from only two representative strains of CA-MRSA.

In conclusion, we found the combination of gemifloxacin and trimethoprim/sulfamethoxazole to be additive or synergistic in most isolates tested. This combination also displayed potent bactericidal activity over 96 h. Gemifloxacin combined with trimethoprim/sulfamethoxazole, clindamycin or rifampicin all prevented the emergence of efflux-mediated resistance to gemifloxacin. While fluoroquinolones are generally not recommended as monotherapy for infections caused by MRSA due to the rapid emergence of resistance reported by numerous other authors and observed in this investigation, these combinations may have utility in the treatment of CA-MRSA infections.

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References


