Trimethoprim-Sulfamethoxazole Activity and Pharmacodynamics against Glycopeptide-Intermediate Staphylococcus aureus

Sandy J. Close, Pharm.D., Christopher R. McBurney, Pharm.D., Cory G. Garvin, Pharm.D., David C. Chen, Pharm.D., and Steven J. Martin, Pharm.D.

Study Objective. To determine the activity of trimethoprim-sulfamethoxazole (TMP-SMX) against glycopeptide-intermediate Staphylococcus aureus (GISA).


Setting. University laboratory.

Measurements and Main Results. Minimum inhibitory concentrations (MICs) of TMP-SMX were determined for three GISA strains. Time-kill assays were conducted at 1 x MIC and at simulated peak serum concentrations (C_max). Two dosing regimens of TMP-SMX were investigated: TMP-SMX 8 mg (TMP)/kg/day and TMP-SMX 15 mg/kg/day, each divided into two doses/day. Both dosages were studied against each strain in a two-compartment in vitro model to determine concentration-related activity. All isolates were susceptible to TMP-SMX. In time-kill studies at 1 x MIC, TMP-SMX was bacteriostatic against all isolates and bactericidal against two of three strains at simulated C_max. The 15-mg/kg/day (divided-dose) regimen provided the best overall reduction in colony-forming units/ml.

Conclusion. All GISA strains were susceptible to TMP-SMX. In addition, it appears that TMP-SMX may have concentration-dependent antibacterial activity against these organisms. As an option in the management of GISA infection, TMP-SMX merits further study.

(Pharmacotherapy 2002;22(8):983–989)
gene. Resistance may be related to thickened cell walls or increased penicillin-binding protein development. The decreased susceptibility of these organisms has been associated with prolonged exposure to vancomycin. With removal of vancomycin, some isolates revert to lower vancomycin MICs.

Several GISA strains with a common ancestral clone have vancomycin MICs of 2–8 µg/ml. Colonies of these strains contain subpopulations with a vancomycin MIC of 8 µg/ml. Thus, a homogeneously resistant population (vancomycin MIC > 8 µg/ml) is easily obtainable by a one-step selection with 4–8 µg/ml of vancomycin. The prevalence of heterogeneously resistant strains may be up to 22% in some hospitals. This partly explains the therapeutic failure of vancomycin in treatment of MRSA infections.

There is no single drug or drug regimen of choice for treatment of GISA infections. Several antibiotics and combination regimens have been used clinically, and many more have been evaluated in vitro. Newer antibiotics, such as quinupristin-dalfopristin and linezolid, show promise as monotherapies, but their effectiveness against GISA has not yet been confirmed in clinical studies. Trimethoprim-sulfamethoxazole (TMP-SMX) has excellent in vitro activity against methicillin-susceptible S. aureus and MRSA. It is an accepted alternative to vancomycin for prevention and treatment of MRSA infections, and several reports document its efficacy against these infections.

Trimethoprim-sulfamethoxazole (TMP-SMX) has excellent in vitro activity against methicillin-susceptible S. aureus and MRSA. It is an accepted alternative to vancomycin for prevention and treatment of MRSA infections, and several reports document its efficacy against these infections.

Materials and Methods

We used three clinical GISA strains in all tests: 992, MU50, and 14379. In addition, S. aureus American Type Culture Collection (ATCC) 29213 was used for quality control. Vancomycin (Sigma, St. Louis, Missouri) and TMP-SMX (TMP Sigma, SMX Sigma) were used for all tests. Mueller-Hinton broth (Difco, Detroit, MI), supplemented with calcium chloride 20 µg/ml and magnesium chloride 10 µg/ml was used for each experiment. Mueller-Hinton agar plates (Remel; Lenexa, KS) were used to maintain the organisms and for colony counts. Mueller-Hinton agar with vancomycin 4 µg/ml was used for selection of the heterogenous strains MU50 and 14379 before each experiment.

MIC Testing

Microdilution broth MICs for vancomycin and TMP-SMX were determined according to guidelines from the National Committee for Clinical Laboratory Standards (NCCLS). The MICs were evaluated before all experiments and at 48 hours after model runs for organisms with residual growth. An inoculum was prepared from bacteria in logarithmic growth to match a 0.5 McFarland standard and then diluted to obtain a final test inoculum of 1.5 x 10^5 colony-forming units (cfu)/ml. Trimethoprim-SMX was tested in a physiologic 1:19 ratio. Concentrations tested were vancomycin 0.0625–128 µg/ml and TMP 0.03125–16 µg/ml–SMX 0.59375–304 µg/ml. For quality control, S. aureus ATCC 29213 was included. Each MIC assessment was performed four times, and the mode value was selected.

Time-Kill Analysis

Time-kill assays were performed for TMP-SMX against each GISA strain according to NCCLS guidelines. We tested TMP-SMX at 1 x MIC and at simulated in vivo peak serum concentrations (C_max; TMP 2.45 µg/ml–SMX 46.55 µg/ml). An inoculum of 1.5 x 10^5 cfu/ml was used for all time-kill assays. The inoculum was prepared by placing 1–2 colonies of an overnight growth of test organisms in Mueller-Hinton broth, incubating at 35°C on a shaking platform, and allowing the organisms to come to logarithmic growth for 3–5 hours. The inoculum was diluted in saline to match the density of a 0.5 McFarland standard using a spectrophotometer (Spectronic 20 Genesys; Milton Roy Co., Rochester, NY). The inoculum was confirmed with colony counts. Organism and antibiotic were added together, and time-kill tubes containing a total volume of 2 ml were incubated at 35°C in room air on a shaking platform at 150 rpm. Samples were removed from each tube at 1, 4, 8, 12, and 24 hours, diluted 1:100 or greater as
necessary in 0.9% sodium chloride to prevent antibiotic carryover, and plated on Mueller-Hinton agar with a spiral dispensing device (Spiral Biotech, Bethesda, MD). All plates were incubated for 24 hours at 35°C in room air. Bacterial colony counts were determined with a laser colony counter (Microbiology International, Rockville, MD) and confirmed by hand counts. The lower limit of detection for this method is 2.3 log_{10} cfu/ml. Reductions in bacteria from baseline of 3 log_{10} cfu/ml or greater were considered to indicate bactericidal activity. All time-kill assays were performed in duplicate, and the mean value (± SD) was determined.

In Vitro Pharmacodynamic Infection Model

An in vitro two-compartment glass infection model was used to simulate human pharmacokinetics. In this model, an outer “central” compartment simulates central blood circulation, and an inner “peripheral” or “infection” compartment simulates tissue infection. Each organism was tested in the model. The inoculum was prepared in a similar fashion to the time-kill experiments. Concentrations of TMP-SMX (1:19 physiologic ratio) simulated the pharmacokinetics of the drug in critically ill trauma patients, a patient population at risk for GISA infection. Doses and dosing intervals for TMP-SMX are not defined clearly. For this reason, we investigated two dosing regimens: TMP-SMX 8 mg (TMP)/kg/day and TMP-SMX 15 mg/kg/day, each divided into two doses/day, which yielded C_{max} of 3.00 µg/ml and 5.67 µg/ml (shown in TMP µg/ml), and trough serum concentrations (C_{min}) of 1.26 µg/ml and 2.38 µg/ml, respectively.23

Bacteria were added to the peripheral compartment, a 10-ml chamber made with a 12,000–14,000 molecular weight cutoff dialysis membrane (Spectra/Por; Spectrum Labs, Rancho Dominguez, CA). This membrane prevents outward migration of the bacteria yet allows passage of antibiotics into the compartment. Antibiotics were injected into the 325-ml central compartment in a bolus fashion over 15–30 seconds. Fresh supplemented Mueller-Hinton broth was introduced to the central compartment with a peristaltic pump (Masterflex; Cole-Parmer Instrument Company, Chicago, IL), at a rate to achieve an antibiotic half-life of 9.6 hours. The model was placed in an incubator at 35°C in room air for the duration of the experiments. Model experiments were run for 48 hours and were performed in duplicate. The mean value (± SD) was selected. Growth controls without antibiotic were performed before the experiments to ensure adequate bacterial growth in the model. The MICs were confirmed on each day of experimentation from the inoculum used in each experiment, and MICs were repeated on any residual growth at 48 hours.

Pharmacodynamic Analysis

Aliquots of 50 µl were removed from both compartments using a sterile syringe and needle at 0, 2, 4, 8, 12, 18, 24, 30, 36, and 48 hours. The aliquots were diluted 1:100 or greater as necessary in 0.9% sodium chloride to prevent antibiotic carryover and plated logarithmically on Mueller-Hinton agar with a spiral plating device. The remaining portions of the samples were frozen at -70°C for analysis of drug concentration. All plates were incubated for 24 hours at 35°C in room air. Bacterial colony counts were determined with a laser colony counter and confirmed by hand counts. The lower limit of detection for this method is 2.3 log_{10} cfu/ml. All pharmacodynamic assays were performed in duplicate, and the mean value (± SD) was used. The difference in colony count reduction and rate of bacterial reduction between the two dosages tested was analyzed using Tukey’s test for multiple comparison. A p value of less than or equal to 0.05 was considered significant.

Pharmacokinetic Analysis

Concentrations of TMP-SMX were determined by removing samples for peak and trough concentrations from both compartments at each dosing interval. Samples were stored at -70°C until analysis. Drug concentrations were determined by bioassay in duplicate with S. aureus ATCC 29213 as the reference organism.

Results

Minimum Inhibitory Concentration Testing

Each of the three GISA strains tested was susceptible to TMP-SMX. The MICs against TMP-SMX and vancomycin are shown in Table 1.

Time-Kill Analysis

Time-kill curves are shown in Figures 1 (simulated C_{max}) and 2 (1 x MIC). Using simulated C_{max} concentrations, bactericidal activity for TMP-SMX was observed against strains 14379 and 992. Bacteriostatic activity
occurred against the MU50 strain. For the 992 strain, TMP-SMX was rapidly bactericidal, achieving colony counts below the lower limit of detection at 4 hours. For GISA 14379, the TMP concentration was 9.8 times the MIC. For GISA Mu50 and 992, the TMP concentration was 39.2 times the MIC. In the 1 x MIC time-kill assays, bacteriostatic effects were evident against all three isolates.

Table 1. Minimum Inhibitory Concentration Data

<table>
<thead>
<tr>
<th>GISA Strain</th>
<th>TMP-SMX MIC (µg/ml)</th>
<th>Vancomycin MIC (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14379</td>
<td>0.25–4.75</td>
<td>8</td>
</tr>
<tr>
<td>992</td>
<td>0.0625–1.1875</td>
<td>8</td>
</tr>
<tr>
<td>Mu50</td>
<td>0.0625–1.1875</td>
<td>8</td>
</tr>
</tbody>
</table>

GISA = glycopeptide-intermediate Staphylococcus aureus; TMP-SMX = trimethoprim-sulfamethoxazole; MIC = minimum inhibitory concentration.

Table 2. Mean ± SD Peak and Trough Central Compartment TMP-SMX Concentrations for Model Runs

<table>
<thead>
<tr>
<th>GISA Strain</th>
<th>Dose (mg)</th>
<th>Peak (µg/ml)</th>
<th>Trough (µg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>14379</td>
<td>8</td>
<td>3.46 ± 0.23</td>
<td>1.20 ± 0.15</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>5.08 ± 0.10</td>
<td>2.33 ± 0.19</td>
</tr>
<tr>
<td>992</td>
<td>8</td>
<td>3.02 ± 0.11</td>
<td>1.12 ± 0.09</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>5.59 ± 0.12</td>
<td>2.33 ± 0.10</td>
</tr>
<tr>
<td>Mu50</td>
<td>8</td>
<td>3.70 ± 0.03</td>
<td>1.35 ± 0.10</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>5.64 ± 0.11</td>
<td>2.39 ± 0.04</td>
</tr>
</tbody>
</table>

TMP-SMX = trimethoprim-sulfamethoxazole; GISA = glycopeptide-intermediate Staphylococcus aureus.

In Vitro Model

The values obtained for peak and trough pharmacokinetic parameters are shown in Table 2. Results of the in vitro model experiments are shown in Figures 3–5. A 1-log difference was observed in inoculum at baseline for the 992 control model as compared with the antibiotic-containing models (Figure 5). However, growth characteristics for the 992 control resembled those of the growth controls of the other isolates during the first 4 hours. Both dosing regimens were bactericidal against the MU50 strain (Figure 1).
3), with organism counts below the limit of detection within 24 hours. For strains 14379 (Figure 4) and 992 (Figure 5), the 15-mg/kg/day regimen provided the best overall reduction in cfu/ml. Colony counts fell below the lower limit of detection by 24 hours with the higher dosing regimen against 992. The TMP-SMX MIC for strain 992 increased to TMP 4 µg/ml–SMX 76 µg/ml at 48 hours (baseline TMP 0.0625 µg/ml–SMX 1.1875 µg/ml) after the 8-mg/kg/day regimen. No changes in MIC were observed for strains 14379 or MU50 at 48 hours. Bacterial killing did not begin in any of the models until 4-8 hours, correlating with peak peripheral compartment drug concentrations. The difference in colony count reduction between the two dosages tested was significant for strain 992 (p=0.03) but not did not reach statistical significance for strains 14379 or MU50.

Discussion

Clinicians have long used TMP-SMX as an alternative to synthetic penicillins or vancomycin in the treatment of S. aureus infection and colonization. Against MRSA, TMP-SMX has demonstrated good clinical efficacy. A study reported an 86% clinical cure rate among 43 patients with MRSA infection treated with TMP-SMX. Another study evaluated the use of TMP-SMX in six patients with osteomyelitis secondary to MRSA. Five patients experienced complete responses, and the sixth discontinued therapy after 2 weeks due to neutropenia. Several in vitro studies have found MRSA strains to be susceptible to TMP-SMX, with the TMP component of MICs ranging from 0.018-0.5 µg/ml. In vitro data for TMP-SMX against clinical GISA strains is also promising, with several isolates found to be susceptible. This agent was used clinically in one reported case of GISA infection. In this case, the GISA isolate (14379) was susceptible to chloramphenicol, rifampin, TMP-SMX, and tetracycline. The patient was treated successfully with oral rifampin 300 mg/day and oral TMP 160 mg/day-SMX 800 mg/day. Other clinical reports document the susceptibility of GISA to TMP-SMX. A report from New Jersey described a GISA isolate (992) susceptible to gentamicin, TMP-SMX, tetracycline, and imipenem. The patient was treated effectively with vancomycin, gentamicin, and rifampin, but eventually died. In the first reported instance of a GISA infection in France, the GISA strain proved susceptible to pristinamycin and TMP-SMX after the patient failed initial treatment with teicoplanin and amikacin. Eradication of the organism occurred with quinupristin-dalfopristin. Recently a GISA infection was reported in Korea. A man with a longstanding MRSA pelvic abscess received long courses of both vancomycin and teicoplanin. He

![Figure 4](image-url) In vitro modeling of TMP-SMX 8 mg (TMP)/kg/day and 15 mg/kg/day, each divided into two doses/day, against glycopeptide-intermediate Staphylococcus aureus 14379. CFU = colony-forming units.

![Figure 5](image-url) In vitro modeling of TMP-SMX 8 mg (TMP)/kg/day and 15 mg/kg/day, each divided into two doses/day, against glycopeptide-intermediate Staphylococcus aureus 992. CFU = colony-forming units. p=0.03, colony count reduction for 8 mg/kg/day versus 15 mg/kg/day.
died as a result of MRSA sepsis, and his blood produced a S. aureus isolate with a vancomycin MIC of 8 µg/ml. The organism was susceptible to rifampin and TMP-SMX but resistant to ciprofloxacin, clindamycin, erythromycin, gentamicin, and tetracycline.5

Our MIC analyses showed all three GISA isolates to be susceptible to TMP-SMX. There are no data to describe the pharmacodynamic parameters that best predict organism eradication in time-kill or other in vitro testing systems with TMP-SMX. Most β-lactams and macrolides exhibit concentration-independent killing, whereas aminoglycosides and fluoroquinolones exhibit concentration-dependent killing. Comparable work has not been performed for TMP-SMX. Both SMX and TMP inhibit key steps in DNA synthesis. Either compound alone is bacteriostatic against most bacteria, but the combination is highly bactericidal against many bacterial species.

In our study, the concentration of drug appeared to play an important role in bacterial killing. In time-kill assays at 1 x MIC, TMP-SMX was bacteriostatic against all three GISA isolates. At simulated peak serum concentrations, TMP-SMX was bactericidal against two of the three strains. Although activity appears to be strain specific, the high drug concentration:MIC ratio may be responsible for the rapid and complete eradication of organisms in these two strains. At simulated peak serum concentrations of 9.8–39.2 x MIC, complete eradication of the organism occurred in two of three strains, with a greater than 2-log cfu/ml colony count reduction in the third strain. This may represent a concentration-dependent effect of the drug against this organism.

Data from the in vitro model seem to confirm this concentration-dependent antibacterial effect. For two of the three isolates tested (992 and 14379), greater colony count reduction was observed with the 15-mg/kg/day dosage than with the 8-mg/kg/day dosage. Both dosages were equally effective against the third isolate (MU50). Development of resistance appeared to be the cause of this discrepancy in isolate 992, with a 6-fold increase in TMP-SMX MIC at 48 hours for the strain exposed to 8 mg/kg/day. The MIC did not change when the isolate was exposed to TMP-SMX 15 mg/kg/day. Similarly, postexposure MICs were unchanged for the other two isolate-dosage combinations. The difference in results between time-kill testing and the model is likely due to the static nature of the time-kill assay.

The role of TMP-SMX in management of infections caused by GISA remains unclear. Advantages of TMP-SMX are its low cost, the extensive clinical experience with its use, and the potential to conserve antibiotics with unique gram-positive activity for situations in which no alternative therapies exist. However, no data are available for TMP-SMX monotherapy in patients with GISA infection, and only one case report describes its use in combination for this purpose.

Conclusion

Our results from MIC testing, time-kill assays, and an in vitro pharmacodynamic model of infection show that TMP-SMX has activity against three GISA isolates. In addition, it appears that TMP-SMX may have concentration-dependent antibacterial activity against these organisms. This agent has been used successfully in patients infected with MRSA and GISA. Further studies should evaluate the activity of TMP-SMX in combination with other antibiotics against GISA, as well as in combination with the human immune system to provide a better prediction of its clinical efficacy.

Acknowledgments

The authors acknowledge the technical advice and counsel of Dr. Diane Cappelletty. The GISA strains tested were kindly provided by Dr. Michael Rybak.

References


