Tissue penetration and antimicrobial activity of standard- and high-dose trimethoprim/sulfamethoxazole and linezolid in patients with diabetic foot infection

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Objectives: The purpose of this study was to conduct a pharmacokinetic and pharmacodynamic evaluation of high (320/1600 mg) and standard (160/800 mg) doses of trimethoprim/sulfamethoxazole and linezolid in outpatients with mild diabetic foot infections (DFIs).

Methods: Both viable skin/soft tissue from the infection site and serum were obtained at various times after antibiotic administration from 18 patients (6 per study group) being treated with linezolid, standard doses of trimethoprim/sulfamethoxazole or high doses of trimethoprim/sulfamethoxazole during a follow-up clinic visit. These samples were assayed for drug concentrations by liquid chromatography in tandem with mass spectrometry. Patient sera were also utilized in time–kill assays against two strains of Staphylococcus aureus and three strains of β-haemolytic streptococci.

Results: The mean tissue/serum ratio for linezolid was 0.46 (range, 0.18–0.71). The mean tissue/serum ratio for trimethoprim was 1.2 (range, 0.3–4.5) for both standard and high doses, and 0.23 (range, 0.1–0.46) and 0.36 (range, 0.14–1.28) for standard and high doses of sulfamethoxazole, respectively. Linezolid exhibited inhibitory activity in time–kill assays against strains of S. aureus (0.45 ± 0.5 log10 cfu/mL) and β-haemolytic streptococci (2.2 ± 0.6 log10 cfu/mL), while trimethoprim/sulfamethoxazole exhibited bactericidal (>3 log kill) activity against all of these isolates. These findings were consistent for each sampling time and for high as well as standard doses of trimethoprim/sulfamethoxazole.

Conclusions: This pharmacokinetic/pharmacodynamic study found that trimethoprim/sulfamethoxazole exhibits good skin/soft tissue penetration in patients with DFIs as well as bactericidal activity in serum against strains of S. aureus and β-haemolytic streptococci.

Keywords: pharmacokinetics, time–kill, diabetes

Introduction

The use of trimethoprim/sulfamethoxazole for the treatment of complicated skin and soft-tissue infections (cSSTIs), including diabetic foot infections (DFIs), has increased dramatically over the past decade due to the increasing prevalence of methicillin-resistant Staphylococcus aureus (MRSA).1,2 Even with this extensive use of trimethoprim/sulfamethoxazole for cSSTIs, there remains uncertainty regarding the activity of this agent against streptococci, the penetration and activity of these antimicrobials in infected skin and soft tissue, and the dosing regimen that provides maximum pharmacodynamic benefits.3 Due to limited efficacy data, recommended options for the treatment of cSSTIs vary and include both standard (160/800 mg) and high (320/1600 mg) doses twice daily of trimethoprim/sulfamethoxazole for MRSA and combination therapy with a β-lactam antibiotic for treatment of β-haemolytic streptococci.4,5

Recent investigations have provided additional insight into the role of trimethoprim/sulfamethoxazole in the treatment of cSSTIs. In a 10 year (2001 – 10) time series analysis, a significant increase in trimethoprim/sulfamethoxazole use was not associated with a significant change in the rates of susceptibility among clinical isolates, including community-associated MRSA.2 Similar findings for MRSA infections have also been reported at the Detroit
Medical Center. Furthermore, recommendations against the use of trimethoprim/sulfamethoxazole for Streptococcus pyogenes infections due to inherent resistance may no longer be valid with the advent of newer testing standards. Clinical strains of S. pyogenes have been shown to be highly susceptible to trimethoprim/sulfamethoxazole in vitro when tested in low-thymidine media. The findings from these susceptibility studies are supported by newer clinical trials in patients with cellulitis and cSSTs, including DFIs. Therapy with trimethoprim/sulfamethoxazole was shown to be as clinically effective as clindamycin and cefalexin in patients with cellulitis and doxycycline in patients with cSSTs due to MRSA. Moreover, in an observational cohort of patients with cSSTs due to MRSA, clinical resolution of infection was not found to be different in patients treated with high doses compared with standard doses of trimethoprim/sulfamethoxazole.

To further complement these clinical trials, we studied the pharmacokinetics and pharmacodynamics of high and standard doses of trimethoprim/sulfamethoxazole in patients with DFIs. These investigations included the measurement of both serum and tissue concentrations of trimethoprim/sulfamethoxazole and an analysis of serum kill curves against common Gram-positive cocci associated with these infections. We also performed similar investigations in patients with DFIs treated with linezolid. Since linezolid has been well studied in this patient population, these findings would be useful for comparative analyses with previous studies with linezolid as well as our observations with trimethoprim/sulfamethoxazole.

Patients and methods

Patients

Ambulatory adult patients with a mild DFI, requiring antibiotic therapy and subsequent surgical debridement, were enrolled into this study. Patients with a history of hepatic or renal failure, allergies to linezolid or sulfonamide antimicrobials or who were pregnant were excluded from this investigation. Study subjects were equally randomized to receive oral linezolid (600 mg orally every 12 h), standard oral doses (160 mg/800 mg every 12 h) of trimethoprim/sulfamethoxazole or high oral doses (320 mg/1600 mg every 12 h) of trimethoprim/sulfamethoxazole for 7 or 14 days as determined by the pediatric surgeon. Specimens for culture were not obtained from these patients. Before entry into this clinical trial, each patient gave written informed consent and the study was approved by the Michigan State University research review committee.

Patients were scheduled to return to either a morning or afternoon clinic, within 1 week after initiating antibiotic therapy, for clinical evaluation and debridement of their infection, to elicit any side effects associated with their antibiotic treatment and to obtain blood and tissue samples.

Serum and tissue samples

At the follow-up clinic visit, which occurred at various times following the most recent dose of antibiotic, a viable skin/soft tissue sample from the infection site was obtained from each patient by a pediatric surgeon. The procurement of these samples occurred at random times after antibiotic administration. These specimens were wiped gently with dry gauze, placed into a pre-weighted vial and stored at –70°C until time of analysis. A venous blood sample was also obtained during this visit. Following centrifugation, serum samples were aliquoted and stored under the same conditions. Both serum and tissue concentrations of linezolid, trimethoprim and sulfamethoxazole were determined at the University of North Texas Health Science Center (Fort Worth, TX, USA) using liquid chromatography (LC)–tandem mass spectrometry (MS/MS).

Calibration samples were prepared by adding various amounts of linezolid into human serum and tissue homogenate (20% w/v in PBS, pH 7.4) obtained from untreated patients. To 50 μL aliquots, sulfamethoxazole-d₄, and trimethoprim-d₃ (both from C/DIN Isotopes, Pointe-Claire, Quebec, Canada) were added as stable-isotope-labelled internal standards (ISs) dissolved in 10 μL of acetonitrile to give IS concentrations of 100 μg/mL and 50 μg/g tissue in serum and tissue, respectively. After brief (10 s) vortexing and the addition of 150 μL of ice-cold acetonitrile, the sample was vortexed again and then sonicated for 10 min. The precipitated proteins were centrifuged off (14,500 rpm, 5 min) and 50 μL of supernatant was removed and diluted with 50 μL of deionized water. Calibration ranges for linezolid in serum and tissue were 0.01–100 μg/mL and 0.12–50 μg/g tissue, respectively. Calibrations for isotope dilution MS/MS to quantify sulfamethoxazole and trimethoprim were performed using a method similar to that described earlier for acetylcarnitine.

Analyses were performed on an LCQ-Deca ion-trap mass spectrometer interfaced to a Surveyor LC system equipped with an autosampler. The manufacturer’s electrospray ionization (ESI) source was operated in the positive-ion mode. Nitrogen was used as both the sheath and auxiliary gas at a pressure of 80 and 20 U, respectively. The spray voltage was set at 5.0 kV and the capillary temperature was 325°C. Collision-induced dissociation (CID) product ion MS/MS spectra were collected using 1.0 Th parent ion isolation width and 46% relative collision energy, with helium used as collision gas. The parent ions of linezolid (m/z 338), sulfamethoxazole (m/z 254), sulfamethoxazole-d₄ (m/z 258), trimethoprim (m/z 291) and trimethoprim-d₃ (m/z 294) were mass-selected for CID and then their principal product ions, m/z 296, 188, 192, 230 and 230, respectively, were monitored [selected-reaction monitoring (SRM)]. Separations were performed using a 100×4.6 mm i.d. Xbridge C18 column packed with 5 μm particles (Waters, Milford, MA, USA) at 1.0 mL/min flow rate and 40°C column temperature. The eluent system consisted of 5 mM aqueous ammonium acetate solution (A) and acetonitrile (B). The elution composition was initially set at 5% B, then was linearly increased after sample injection to 95% B in 6 min and held for 0.1 min; finally, the mobile phase composition was retained at 0.1 min to 5% B to equilibrate for 2 min before the subsequent run. The injection volume was 10 μL. The effluent was diverted to waste for 1.8 min after injection to reduce the amount of salt entering the ESI source.

Data acquisition and processing were performed by the manufacturer’s XCalibur (version 2.0) software. For linezolid, quadratic calibration was employed by taking the ratio of the integrated linezolid and trimethoprim-d₃ (IS) SRM traces of the LC–MS/MS runs. Quantitative analyses of sulfamethoxazole and trimethoprim utilized the principle of isotope-dilution LC–MS/MS, which was performed from the SRM traces using a method similar to that described earlier for acetylcarnitine.

Time–kill curves

Isolates of S. aureus and β-haemolytic streptococci (groups A and B) were used to analyse the antimicrobial activity of the study antibiotics in serum (Table 1). The MICs of linezolid, trimethoprim and sulfamethoxazole were determined by microdilution methods according to the CLSI (formerly NCCLS). Each serum sample was tested against these bacteria by a modification of the time–kill method described in the CLSI guidelines. Two hundred and twenty-five microtiter of each serum sample was plated on microtitre plates and inoculated with the bacterial strains. To prepare the inoculum, staphylococcus colonies were suspended in cation-supplemented Mueller–Hinton broth and streptococcus colonies were suspended in cation-supplemented Mueller–Hinton broth with 5% lysed horse blood. Twenty-five microtiter of inoculum was used to inoculate each serum sample. Viability counts of each culture were carried out at 0, 2, 6 and 24 h after inoculation. Sampling was done by removing a 5 μL aliquot from each sample and serially diluting them 10-fold in media to minimize
antibiotic carryover. Ten microlitre aliquots of both the undiluted and diluted samples were plated on Mueller–Hinton plates (staphylococci) or blood agar plates (streptococci) and colonies were counted after 24 h of incubation at 35 °C. Time–kill assays were analysed by determining the number of bacteria (log10 cfu/mL) at 2, 6 and 24 h compared with counts at 0 h. The range of quantification was 20–200 cfu/mL. Growth controls were included in each experiment. Geometric means (log10 cfu/mL) were used to determine the difference in log kill at 24 h.

Results

A total of 18 patients (6 per treatment group) were enrolled into this study. All had diabetes and infected foot ulcers that were being treated by a local podiatrist (J. K. T.). The demographics of these patients are presented in Table 2. All study subjects returned to the clinic for an evaluation 2–7 days after initiation of antimicrobial therapy. These patients were compliant with the prescribed

<table>
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<tr>
<th>Isolate</th>
<th>Linezolid MIC (mg/L)</th>
<th>Trimethoprim/sulfamethoxazole MIC (mg/L)</th>
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<tr>
<td>Group B streptococci (ATCC 49446)</td>
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MSU, Michigan State University; JMI, JMI Laboratories.

*aLinezolid susceptibility per CLSI ≤2 mg/L (β-haemolytic streptococci) and ≤4 mg/L (S. aureus).

*bTrimethoprim/sulfamethoxazole susceptibility breakpoint for S. aureus ≤2/38 mg/L (CLSI). CLSI does not provide susceptibility breakpoints for β-haemolytic streptococci. The EUCAST breakpoint is ≤1 mg/L.

Table 2. Demographics and antibiotic concentrations in the three treatment groups

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<th>Sex</th>
<th>Age (years)</th>
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<th>Sampling time (h)</th>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>serum (mg/L)</td>
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<td>3.9/5.7</td>
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<td>38.5±12</td>
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<td>Trimethoprim/sulfamethoxazole (320 mg/1600 mg)</td>
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<td>45/311.5</td>
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<td>127</td>
<td>34</td>
<td>10</td>
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<td>98±31</td>
<td>33.5±6.5</td>
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</tbody>
</table>

BMI, body mass index.

*aHours following antibiotic administration.
antibiotic treatment and their infections were responding to treatment, with the exception of one patient receiving standard doses of trimethoprim/sulfamethoxazole, who was subsequently found to have osteomyelitis. No medication side effects were reported by subjects receiving linezolid or standard doses of trimethoprim/sulfamethoxazole, but two patients complained of stomach upset while taking high doses of trimethoprim/sulfamethoxazole.

Blood and tissue were obtained 1–12 h after linezolid administration (Table 2). In this study group, serum concentrations ranged from 34 mg/L (2 h) to 7 mg/L (10 h) and tissue concentrations ranged from 15.2 μg/g (2 h) to 3.5 μg/g (10 h). The mean tissue to serum ratio was 0.46 (range, 0.18–0.71) which is similar to that found in our previous study of diabetic patients with similar characteristics.15 In patients receiving standard doses of trimethoprim/sulfamethoxazole, blood and tissue were obtained 1–8 h after antibiotic administration (Table 2). The sulfamethoxazole and trimethoprim serum concentrations as well as the mean ratio of sulfamethoxazole to trimethoprim (24.4; range, 20–31.8) were comparable to those found in previous pharmacokinetic investigations.16 The mean tissue to serum ratios of trimethoprim and sulfamethoxazole were 1.2 (range, 0.4–2.2) and 0.23 (range, 0.1–0.46), respectively. The mean tissue/tissue ratio

**Figure 1.** Antibacterial activity of standard-dose (SD) and high-dose (HD) trimethoprim/sulfamethoxazole (TMP/SMX) and linezolid against (a) methicillin-susceptible S. aureus (ATCC 29213) and (b) MRSA (MSU 33).

**Figure 2.** Antibacterial activity of standard-dose (SD) and high-dose (HD) trimethoprim/sulfamethoxazole (TMP/SMX) and linezolid against (a) group A streptococci (ATCC 11436), (b) group A streptococci (JMI 32695) and (c) group B streptococci (ATCC 49446).
of sulfamethoxazole to trimethoprim was found to be 6.7 (range, 1.5–13). The high-dose trimethoprim/sulfamethoxazole treatment group had similar mean serum (20.5; range, 11.5–33.5) and tissue (6.3; range, 4.7–9.1) ratios of sulfamethoxazole to trimethoprim compared with the standard-dose treatment group. The mean tissue to serum ratios in the high-dose patient group for trimethoprim (1.2; range, 0.3–4.5) and sulfamethoxazole (0.36; range, 0.14–1.28) were also comparable to the calculated ratios from the standard-dose patient group.

The mean log kill in serum from patients receiving linezolid was $0.45 \pm 0.5 \log_{10} \text{cfu/mL}$ (range, 0.1–1.3 $\log_{10} \text{cfu/mL}$) at 24 h against the two strains of $S. aureus$ (Figure 1). The magnitude of the log kill for these six sera was independent of sampling time. In contrast, the serum from each patient who received trimethoprim/sulfamethoxazole, both standard and high doses, produced $>3$ log kill at 24 h against both isolates of $S. aureus$ (Figure 1). Linezolid exhibited greater log kill against $\beta$-haemolytic streptococci compared with $S. aureus$ (Figure 2). Log kill was once again independent of sampling time and exhibited a mean of $2.2 \pm 0.6 \log_{10} \text{cfu/mL}$ (range, 1.4–3.9 $\log_{10} \text{cfu/mL}$) against both group A and group B streptococcal strains. As observed with $S. aureus$, both doses of trimethoprim/sulfamethoxazole produced $>3$ log kill at 24 h for patient sera against $\beta$-haemolytic streptococci independently of sampling time. This finding also includes the group A strain that was ‘non-susceptible’ to trimethoprim/sulfamethoxazole (Figure 2b).

Discussion

The serum and tissue concentrations of an antibiotic are important factors that help determine clinical efficacy. Physiological changes, such as decreased bioavailability and local inflammation, can affect the serum and tissue pharmacokinetics of antimicrobial agents in patients with diabetes, leading to impaired target-site penetration. Tissue penetration is, thus, an important determinant of antibiotic activity and therapeutic outcome. Adequate serum concentrations of a particular antibiotic do not necessarily result in detectable concentrations in the ischaemic tissue of the diabetic foot. The serum concentrations of trimethoprim and sulfamethoxazole measured in our study patients are comparable to those obtained in studies of healthy subjects. The skin/soft tissue concentrations of these agents in DFIs have not been reported previously. We observed mean tissue concentrations that were similar to serum concentrations for trimethoprim and 30% of serum concentrations for sulfamethoxazole. Our study design resulted in various sampling times after antibiotic administration and the range of these times was similar for both standard and high doses of trimethoprim/sulfamethoxazole. Furthermore, the variability in the penetration ratios was not a function of sampling time. Although the whole-tissue concentrations of trimethoprim/sulfamethoxazole reported in this study represent both extracellular and intracellular concentrations and may not depict the concentration of drug at the site of infection, these concentrations and penetration ratios are comparable to those reported in skin blister, a biological matrix believed to approximate the concentration of antibiotic in the interstitial space. The tissue to serum ratio of 46% observed with linezolid was close to the ratio of 51% we observed in a similar study using parenteral linezolid in the treatment of hospitalized patients with DFIs.

Numerous studies have assessed the microbiology of DFIs, and virtually all show that aerobic Gram-positive cocci, particularly $S. aureus$ and $\beta$-haemolytic streptococci, cause most acute infections. The rise of MRSA in DFIs has led to an increased use of trimethoprim/sulfamethoxazole in ambulatory patients with mild to moderate infections. Community-acquired strains of $S. aureus$ are usually susceptible to trimethoprim/sulfamethoxazole but $\beta$-haemolytic streptococci exhibit variable susceptibility. Much of the previously reported resistance in $\beta$-haemolytic streptococci was likely due to the methodology of testing those strains. A high thymidine content in agar provides an exogenous substance that can be used by selected bacteria to maintain folate metabolism, so that they appear resistant to trimethoprim/sulfamethoxazole. Recent susceptibility experiments with clinical isolates of $S. pyogenes$ have shown that resistance rates are very low when the thymidine content of the growth media is strictly regulated. Thymidine can also be associated with clinical failure of trimethoprim/sulfamethoxazole due to its release from a large bacterial inoculum or inflamed tissues. A major component of pus is polymerized DNA that is released from inflammatory cells and injured tissues. $S. aureus$ thermonuclease can release thymidine from DNA, which, in turn, antagonizes the antistaphylococcal effects of trimethoprim/sulfamethoxazole. This discovery adds further evidence supporting incision and drainage of abscesses prior to treatment with trimethoprim/sulfamethoxazole.

In our time–kill experiments, we tested the serum concentrations of these antimicrobials at various times after administration. The use of patient serum allows one to integrate antimicrobial activity with pharmacokinetic parameters. Moreover, these time–kill studies occur in the presence of serum factors such as antibodies, complement and protein binding at actual drug concentrations. The presence of these serum factors can improve the clinical relevance of time–kill data. Previous studies have observed that both trimethoprim/sulfamethoxazole and linezolid exhibit time-dependent ($T_{\text{MIC}}$) antimicrobial activity. This bacterial effect was also observed in our time–kill studies. We observed a similar log kill at 24 h for both antimicrobials against each of the bacterial strains utilized in this study at each of the timepoints tested. Furthermore, serum concentrations from high doses of trimethoprim/sulfamethoxazole did not exhibit greater log kill than those from standard doses of trimethoprim/sulfamethoxazole. Linezolid and trimethoprim/sulfamethoxazole exhibited bacteriostatic and bactericidal activity, respectively, against these strains of $S. aureus$ and $\beta$-haemolytic streptococci. These findings are also consistent with other time–kill studies. Of note, bactericidal activity ($>3$ log kill) at 24 h was also observed at each timepoint for both doses of trimethoprim/sulfamethoxazole against a ‘non-susceptible’ strain of $S. pyogenes$ (Figure 2). This finding was unexpected and possibly due to the presence of serum in these time–kill experiments.

One concern with the widespread use of trimethoprim/sulfamethoxazole is the many associated untoward effects, especially allergic reactions. Moreover, among commonly prescribed antimicrobials, trimethoprim/sulfamethoxazole is associated with a high rate of emergency department visits. All of the patients in our study completed their course of antibiotic therapy, but two subjects who received high-dose trimethoprim/sulfamethoxazole complained of stomach upset. Upper gastrointestinal symptoms (3%–8%) are the most commonly encountered adverse effects with trimethoprim/sulfamethoxazole and...
have generally been attributed to the sulphonamide portion of this agent.\textsuperscript{27} Other toxicities associated with trimethoprim/sulfamethoxazole include hyperkalaemia, haematological disorders, acute kidney injury and delirium.\textsuperscript{27,30,31}

Conclusions

In this study, we observed good tissue penetration of both trimethoprim and sulfamethoxazole, which lends further support for their combined use in the treatment of DFIs. In addition, our time–kill experiments exhibited bactericidal activity for trimethoprim/sulfamethoxazole against both methicillin-susceptible and methicillin-resistant strains of S. aureus as well as group A and group B streptococci. These findings corroborate recent in vitro studies of trimethoprim/sulfamethoxazole activity and suggest that additional antibiotic therapy is unnecessary for empirical treatment of β-haemolytic streptococci. Furthermore, high-dose trimethoprim/sulfamethoxazole did not exhibit greater antimicrobial activity than standard doses in time–kill experiments and was associated with gastrointestinal upset. Our observations with linezolid were similar to those in previous trials and continue to support its use in treatment of DFIs.

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Transparency declarations

None to declare.

References