Serum concentration of co-trimoxazole during a high-dosage regimen

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Objectives: Sulfamethoxazole and trimethoprim have been used for decades, yet high dosages are rarely reported. We aimed to measure blood concentrations of both molecules in this situation.

Methods: Between 2002 and 2010, 22 patients received two tablets of co-trimoxazole three times a day, equivalent to a daily dosage of 2400 mg of sulfamethoxazole and 480 mg of trimethoprim. The trimethoprim and sulfamethoxazole concentrations were determined 3 h after administration using ion-paired HPLC.

Results: In the presence of a negative control, which yielded no peaks at the retention times for trimethoprim and sulfamethoxazole, the mean ± SD value for sulfamethoxazole concentration was 161.01 ± 69.154 mg/L and the mean ± SD value for trimethoprim was 5.788 ± 2.74 mg/L.

Conclusions: These concentrations are largely above the trimethoprim and sulfamethoxazole MIC distributions as well as the trimethoprim resistance clinical breakpoint (4 mg/L) reported by EUCAST in 2012 for most bacterial pathogens, including Gram-positive species such as Staphylococcus aureus. Our results support proposing a high-dosage regimen of co-trimoxazole as a suitable alternative for methicillin-resistant S. aureus infections.

Keywords: sulfamethoxazole, trimethoprim, MRSA

Introduction

Sulphonamide compounds have been used as antibiotics since the 1930s and trimethoprim was first used 30 years later.1 Sulfamethoxazole and trimethoprim both interfere with folate metabolism and the 1:5 trimethoprim/sulfamethoxazole combination known as co-trimoxazole is a broad-spectrum antimicrobial agent used to treat infections due to aerobic Gram-positive and Gram-negative bacteria, fungi and protozoa.2 Co-trimoxazole is available in oral and intravenous preparations with the standard single-strength tablet containing 80 mg of trimethoprim combined with 400 mg of sulfamethoxazole.

Trimethoprim/sulfamethoxazole concentrations have been measured in the serum of patients receiving oral co-trimoxazole equivalent to 160 mg of trimethoprim and 800 mg of sulfamethoxazole daily (Figure 1).1–5 However, a clinical benefit of higher dosage co-trimoxazole regimens has been demonstrated in patients suffering opportunistic infections during human HIV coinfection, including patients diagnosed with Pneumocystis jirovecii infection.6 We previously showed the efficacy of a high-dosage co-trimoxazole regimen for treating patients diagnosed with Staphylococcus aureus osteitis.7 However, the actual co-trimoxazole serum concentration has not been reported in patients receiving a high-dosage regimen of co-trimoxazole for several days.

Methods

Between 2002 and 2010, 22 patients received two tablets of co-trimoxazole three times a day, equivalent to a daily dosage of 2400 mg of sulfamethoxazole and 480 mg of trimethoprim, as previously described.7 In these patients, serum specimens were routinely collected for co-trimoxazole concentration determination after ≥4 weeks of treatment. Venous blood samples were collected 3 h after administration of drugs and the serum was immediately separated from blood cells by centrifugation at 1500 g for 10 min and frozen at −20°C until analysis. Previously, plasma samples containing co-trimoxazole were found to be stable ≥24 h when the samples were kept at room temperature (percentage difference <15%).8

A negative control serum was obtained from a voluntary healthy person who had not received medication for >4 weeks. All patients gave written informed consent for the study and the study was approved by the local institute Fédératif de Recherche IFR48 Ethics Committee. HPLC-grade analytical reagents were obtained from Sigma–Aldrich (Saint-Quentin, France). The trimethoprim and sulfamethoxazole concentrations were determined using ion-paired HPLC. The HPLC equipment consisted of a Merck L6200 pump and Merck UV detector L4000 (Merck, Darmstadt, Germany). The analytical column was a Merck Lichrospher RP18 (5 μm, 125 × 4 mm). The mobile phase consisted of phosphate buffer (0.1 M, pH 4.5) mixed at an 86:14 (v/v) ratio with acetonitrile. The flow rate was 1.3 mL/min and...
detection was monitored at 230 nm. The retention times were 12 ± 0.5 and 23 ± 1.2 min for trimethoprim and sulfamethoxazole, respectively. A 100 μL volume of trichloroacetic acid was mixed with 1 mL of serum, the mixture was vortexed and then the mixture was centrifuged at 1500 g for 10 min. A 50 μL volume of supernatant was injected directly onto the HPLC column. The negative control was tested in parallel to the patient specimens in each analysis. In this analytical procedure, the detection limits were defined as the amount of drug that gave a peak height that was three times the peak-to-peak noise.

Results and discussion

In the presence of a negative control, which yielded no peaks at the retention times for trimethoprim and sulfamethoxazole, the detection limit was 0.025 mg/L for trimethoprim and 0.1 mg/L for sulfamethoxazole, as determined using standards. The range of concentrations tested was 1.0–30 mg/L for trimethoprim and 25–300 mg/L for sulfamethoxazole. The mean ± SD value for the sulfamethoxazole concentration was 161.01 ± 69.154 mg/L and that for trimethoprim was 5.788 ± 2.74 mg/L (Figure 2).

We herein used a simple HPLC method previously developed for the determination of sulfamethoxazole and trimethoprim concentrations in clinical samples and pharmaceutical preparations.3,4,9 This method is more suitable than gas chromatography for routine use in the clinical laboratory, due to the easiness of sample preparation using a sole and simple extraction procedure allowing the simultaneous determination of trimethoprim and sulfamethoxazole concentrations.10 Because the current trimethoprim/sulfamethoxazole 1 : 5 ratio reportedly yields an average serum concentration ratio of 1:20 in vivo,11,12 it was necessary to use a detection system that was more sensitive and precise for trimethoprim in order to obtain a dosage of a similar magnitude for both compounds. The optimum 230 nm UV wavelength used in the present study was selected on the basis of its higher sensitivity for trimethoprim and less serum interferences. The same UV wavelength had been used previously for the determination of trimethoprim, sulfamethoxazole and N4-acetyl sulfamethoxazole in serum and urine.6 A previous method that used a UV wavelength of 225 nm resulted in sulfonamide peaks that were too large in relation to trimethoprim peaks.4 A higher 280 nm UV wavelength yielded less plasma interferences, but a decreased, unsatisfactory sensitivity for trimethoprim concentration determination.10 The simplicity of the preparation, avoidance of extraction procedures and increased sensitivity all make the method used herein appropriate for the analysis of large series of samples. Previously reported determinations of co-trimoxazole have been performed in the blood of patients receiving a daily dosage of 160 mg of trimethoprim and 800 mg of sulfamethoxazole orally or 80 mg of trimethoprim and 400 mg of sulfamethoxazole intravenously.13 In the present work, we observed that an increased dosage of 480 mg of trimethoprim and 2400 mg of sulfamethoxazole resulted in a higher, proportional serum concentration of both molecules with excellent correlation coefficients ($R^2 > 0.872$ for trimethoprim and $R^2 > 0.802$ for sulfamethoxazole). These concentrations are largely above the trimethoprim and sulfamethoxazole MIC distributions as well as the trimethoprim resistance clinical breakpoint (4 mg/L) reported by EUCAST in 2012 for most bacterial pathogens, including Gram-positive species such as S. aureus (http://www.eucast.org/clinical_breakpoints/).14–16 Moreover, since the tissue concentration of trimethoprim is usually higher than the serum concentration,17 our results support proposing a high-dosage regimen of co-trimoxazole as a suitable alternative for
methicillin-resistant *S. aureus* infections. Although the oldest family of antibiotics, sulphonamides should not be forgotten and their continuous supply should be ensured, as they offer alternative treatments with molecules whose concentrations can be monitored for infections resistant to first-line protocols.

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**Transparency declarations**

None to declare.

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