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Original Research

Concentrations of Sulfadiazine and Trimethoprim in Blood and Endometrium of Mares After Administration of an Oral Suspension

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ABSTRACT

The objective of this experiment was to assess the concentrations of sulfadiazine and trimethoprim in the blood and endometrium of nonpregnant mares after oral treatment. We hypothesized that the potentiated sulfonamide would reach tissue concentrations greater than the minimum inhibitory concentration (MIC) reported for common pathogens. Over two breeding seasons, mares in estrus were treated with sulfadiazine-trimethoprim (Equisul-SDT Aurora Pharmaceutical, LLC, Northfield, MN), 333 mg/67 mg combination per mL, at a dosage of 24 mg/kg, orally, every 12 hours for five treatments. Blood was obtained at 0, 12, 36, and 60 hours. An endometrial biopsy was also performed at 60 hours. In year 1, the mean concentrations of sulfadiazine and trimethoprim at 60 hours were 12.14 µg/mL and 0.25 µg/mL in the blood and 3.19 µg/g and 0.69 µg/g in the endometrium, respectively. In year 2, the mean concentrations of sulfadiazine in the blood were 5.17, 10.22, and 13.39 µg/mL and 0.04, 0.15, and 0.27 µg/mL for trimethoprim at 12, 36, and 60 hours, respectively. Mean concentrations of sulfadiazine and trimethoprim in the endometrium at 60 hours were 7.96 µg/g and 0.23 µg/g, respectively. Concentrations of sulfadiazine and trimethoprim in the endometrium after five consecutive treatments with the oral suspension were above the in vitro MIC reported for common pathogens known to cause bacterial endometritis, for example, *Streptococcus equi* subsp. *zooepidemicus* (MIC = 0.25–4 µg/mL) and *Escherichia coli* (>0.25–4 µg/mL). The oral suspension of sulfadiazine-trimethoprim should be an efficacious and viable treatment for bacterial endometritis.

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1. Introduction

Endometritis remains a very important clinical condition in the equine industry and has been ranked as the third most important medical problem of adult horses [1]. Although the treatment for endometritis consists of various approaches [2–5], the treatment of choice for bacterial endometritis is antibiotics [6], typically administered through the intrauterine route [6,7]. However, systemic antibiotics may be preferred over intrauterine antibiotics for equine endometritis for various reasons, including decreased risk of

iatrogenic contamination, ability to treat irrespective of the stage of the estrous cycle, and homogeneous distribution of the antibiotic throughout the endometrium [6–8]. The latter reason is especially relevant, given the increasing evidence that chronic bacterial infections may remain in the deep layers of the endometrium. Nielsen [9] and others have found that endometrial biopsies have higher diagnostic sensitivity than a swab, in which the sample is only taken from the superficial endometrium. Moreover, Petersen et al [10] have shown by fluorescent in situ hybridization that *Streptococcus zooepidemicus* has the capability to chronically reside in the deep endometrium, which could make the bacterium invulnerable to an antibiotic infused into the uterine lumen that would not penetrate deep enough into the endometrium [5,6].

Antibiotics that are often used for endometritis include amikacin, gentamicin, penicillin, ampicillin, ceftiofur sodium, enrofloxacin, and potentiated sulfonamides [6,7]. Sulfonamides are inexpensive antibiotics that acquire bactericidal activity when in very high doses or when combined (potentiated) with diaminopyrimidines, such as trimethoprim [11]. Sulfonamides, in

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combination with trimethoprim, are considered to have a good and moderate susceptibility index against *S. zooepidemicus* and *Escherichia coli*, respectively [11]. These bacteria are considered the first and second most commonly isolated organisms from cases of infectious endometritis [12,13]. Sulfamethoxazole-trimethoprim has been shown to be effective in preventing abortion in mares with experimentally induced placentitis [14]. It has also been demonstrated that the same antibiotic combination reaches therapeutic concentrations in the allantoic fluid of mares in late gestation [15]. Despite the clinical significance of these findings, to the authors' knowledge, there has been a paucity of published data on the uterine tissue distribution of sulfonamides in nonpregnant mares since the late 1980s [16–18]. The recent commercial release of a potentiated sulfonamide for oral administration in horses has prompted the investigation of blood and tissue levels in estrual mares, a period when mares are typically treated for endometritis.

We hypothesized that the administration of an oral formulation of sulfadiazine-trimethoprim would reach concentrations in the endometrium considered therapeutic for *S. zooepidemicus* and *E. coli*. The objective of the study was to determine the concentrations of sulfadiazine and trimethoprim in the peripheral blood and endometrium of mares after treatment during estrus.

2. Materials and Methods

2.1. Animals

These studies were conducted during the breeding seasons over two consecutive years at the Louisiana State University (LSU), Baton Rouge, LA (years 1 and 2), and the University of Maine, Orono, ME (year 1). Animal use was approved by the Institutional Animal Care and Use Committees of each university. A total of forty-one ($n = 41$) mares, weighing an average of 525 kg in year 1 and 499 kg in year 2 were used for the experiments. Over the course of the studies, mares received hay and water ad libitum in addition to commercial concentrate feed. The mares were kept in pastures except during treatment periods, when they were housed in box stalls.

2.2. Mare Selection

Endometrial biopsies obtained from cycling mares were evaluated to confirm that mares were free of endometrial inflammation as per histological evaluation, based on a lack of neutrophilic infiltration in the endometrial epithelium and absence of lymphocytic foci within the strata compacta, and minimal fibrosis. In a subsequent estrous cycle, transrectal palpation and ultrasonography were performed at least three times per week. Treatment with sulfadiazine-trimethoprim was started when mares showed uterine edema and a follicle ≥ 30 mm in diameter by ultrasonographic examination. If a mare had a follicle ≥ 30 mm in diameter and no uterine edema, treatment was started if she displayed signs of behavioral estrus when exposed to a stallion. Any degree of intra-uterine fluid on uterine ultrasound precluded the use of the mare in the study. Variations in the treatment route and samples collected between the two years of the experiment are described below. During and after administration of the sulfadiazine-trimethoprim, mares were observed at least twice daily for any changes in appetite, fecal consistency, or frequency of bowel movements.

2.2.1. Year 1

Seventeen mares from the LSU (aged 6 to 20 years; median, 13 years) and four from the University of Maine (ages 7 to 16 years; median, 13 years) underwent five treatments with a suspension of sulfadiazine-trimethoprim (Equisul-SDT; Aurora Pharmaceutical,

LLC, Northfield, MN; 333 mg/67 mg combination per mL), at a dosage of 24 mg sulfadiazine/kg administered per os every 12 hours. Blood samples and endometrial biopsies were obtained at 60 hours after starting treatment.

2.2.2. Year 2

Twenty mares from the LSU (aged 3 to 18 years; median, 11 years) were treated with the same dosage as in year 1 although for these mares, the drug was administered every 12 hours for five treatments by nasogastric gavage to reduce potential variation between mares. In each administration, the volume of medication was inserted in the nasogastric tube and followed by 2 L of water to ensure ingestion of the complete amount administered. Blood samples were obtained at 0, 12, 36, and 60 hours. An endometrial biopsy was collected at 60 hours.

2.3. Sample Handling and Drug Analyses in Blood and Endometrial Samples

Plain (serum) and heparinized (plasma) 10 mL blood tubes were centrifuged at $3,000 \times g$ after plain blood tubes were allowed to clot at room temperature. Serum and plasma samples were stored at -20°C until analysis. Endometrial biopsies were obtained aseptically in routine fashion [19] from the base of either uterine horn with a uterine biopsy forceps. In year 1, the tissue samples were transferred to a cryovial and kept in crushed ice until transfer to liquid nitrogen (<20 minutes). In year 2, the samples were snap frozen in liquid nitrogen immediately after tissue biopsy. All endometrial samples were stored at -80°C until analysis.

Concentrations of sulfadiazine and trimethoprim in both blood and tissue samples were determined by liquid chromatography tandem mass spectrometry/mass spectrometry (LC-MS/MS). The assays were conducted at the K.L. Maddy Equine Analytical Chemistry Laboratory, UC Davis, CA. Briefly, approximately 100–300 mg of endometrial tissue was weighed into 7-mL Precellys hard tissue homogenizing vials (WIS Biomed, San Mateo, CA), and 4 mL of ACN:1M acetic acid (9:1, v:v) containing 30 ng/mL of d9-trimethoprim and d4-sulfadiazine internal standard was added to all samples. Samples were homogenized with a Precellys 24 tissue homogenizer (Bertin Technologies, Rockville, MD) twice at 6,500 rpm for 60 seconds with a 10 minute cool down period (-20°C) before and after homogenization. The homogenate (1 mL) was aliquoted into autosampler vials and centrifuged in a Sorvall ST 40R centrifuge (Thermo Scientific, San Jose, CA) at 4,300 rpm/4,031 g for 10 minutes at 4°C , and 10 μL of this was injected into the LC/MS system.

2.4. Statistical Analysis

Associations between years and within individual mares were analyzed using a mixed analysis of variance model with location, time, and year as fixed effects and mare as random effect. A Pearson product-moment correlation test was used to measure the strength of association of the relative concentrations of antibiotics in the plasma and endometrium; correlations were estimated by Pairwise method.

3. Results

There were no adverse effects observed as a result of the treatment. There was no effect of location on drug concentrations ($P = .36$). Therefore, data from the two locations (University of Maine [Maine] and LSU) during year 1 were combined. Trimethoprim quantification in endometrial samples of "LSU" location for year 1 were subject to unidentified error during LC-MS/MS assay, and assays could not be repeated; thus, only "Maine" data are available for endometrial trimethoprim from year 1. Concentrations

of sulfadiazine in serum and plasma in year 1 had a very high correlation coefficient ($r = 0.990$). Consequently, in year 2, only blood plasma samples were collected. For clarity of results, serum and plasma samples will be referred to as “peripheral blood” sulfadiazine and trimethoprim.

In year 1, the mean concentrations of sulfadiazine and trimethoprim at 60 hours were $12.14 \pm 0.74 \mu\text{g/mL}$ and $0.25 \pm 0.09 \mu\text{g/mL}$ in the peripheral blood and $3.19 \pm 0.26 \mu\text{g/g}$ and $0.69 \pm 0.23 \mu\text{g/g}$ in the endometrium, respectively (Fig. 1). The respective correlations between concentrations of sulfadiazine and trimethoprim in the peripheral blood and endometrium were $r = 0.898$ and $r = 0.936$ ($P < .001$), respectively.

In year 2, concentrations of antibiotics in the blood increased with time during treatment. The mean concentrations of sulfadiazine in the blood were 5.17 ± 0.34 , 10.22 ± 0.64 , and $13.40 \pm 0.72 \mu\text{g/mL}$ and those of trimethoprim were 0.04 ± 0.01 , 0.15 ± 0.03 , and $0.28 \pm 0.04 \mu\text{g/mL}$ at 12, 36, and 60 hours, respectively (Fig. 2). Endometrial concentrations of sulfadiazine and trimethoprim at 60 hours were $7.96 \pm 0.47 \mu\text{g/g}$ and $0.23 \pm 0.03 \mu\text{g/g}$, respectively (Fig. 3). The correlation coefficients between blood and endometrial tissue concentrations of sulfadiazine and trimethoprim were $r = 0.819$ and $r = 0.948$ ($P < .0001$), respectively.

4. Discussion

The minimum inhibitory concentration (MIC) for sulfadiazine and trimethoprim reported in the literature for *S. zooepidemicus* [11,20,21] and *E. coli*, in vitro, ranges from 0.25 to 4 $\mu\text{g/mL}$ [11,21]. The concentrations in the peripheral blood after treatment with the combined sulfadiazine and trimethoprim were above those MICs at all the time points examined. However, MICs are determined in vitro using the planktonic form of bacteria, and it is not clear how this might relate to bacteria in biofilm or in a dormant state [10]. There was also a pattern of increasing concentrations in the peripheral blood as time progressed after initiation of treatment from 0 hours through 60 hours. The combined sulfadiazine-trimethoprim concentrations in the endometrium at 60 hours were above the higher end of the MIC range in year 2 but did not reach the higher end value of the MIC range in all mares though the combined concentration (3.89 $\mu\text{g/g}$) is approximate to the high end of the MIC range. There is evidence that antibiotic efficacy may be achieved at concentrations less than 4.0 $\mu\text{g/g}$. For example, it has been reported that a concentration of 2.5 μg is bactericidal for *S. zooepidemicus* isolates cultured specifically from samples from mares afflicted with bacterial endometritis [20]. However, the

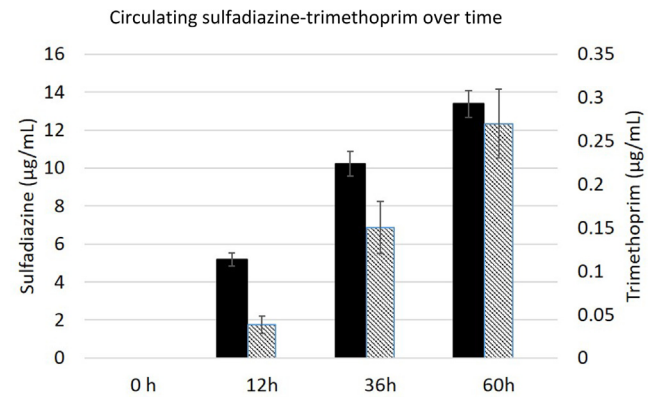


Fig. 2. Concentrations of sulfadiazine ($n = 20$) and trimethoprim ($n = 20$; mean \pm standard error of the mean) in peripheral blood at 12, 36, and 60 h after beginning of treatment during year 2. Sulfadiazine is represented by the solid black bars. Trimethoprim is represented by the dashed bars.

question remains as to why the tissue concentrations were lower in year 1 than in year 2. The concentrations of antibiotics in the blood in years 1 and 2 indicate that the mode of administration (per os vs. oral gavage) did not affect antibiotic absorption. In fact, the variation across mares was lower in the mares given the antibiotic per os, and those mares had a higher correlation between peripheral blood and endometrial concentrations. Perhaps the difference in methodology between years 1 and 2 may explain the lower concentrations of endometrial sulfadiazine in year 1 because endometrial samples were placed into crushed ice before storage in liquid nitrogen. Endometrial samples in year 2 were snap frozen in liquid nitrogen immediately after collection.

More extensive reviews on treatments for endometritis [6,7] have stressed the general lack of data in the literature regarding clinical efficacy of systemic antibiotics as a treatment for bacterial endometritis. Extrapolation for clinical applicability through this route of administration is then made by comparing bioavailability as blood and tissue concentrations to MICs were reported during in vitro testing. Likewise, the present study is limited in a way that the potential efficacy of the sulfadiazine-trimethoprim oral solution is inferred from the MICs that are determined for common uterine bacterial pathogens determined in vitro. A goal of future investigations would be to determine the in vivo efficacy of this sulfadiazine-trimethoprim formulation in mares with bacterial endometritis. The robust demonstration in the present study of

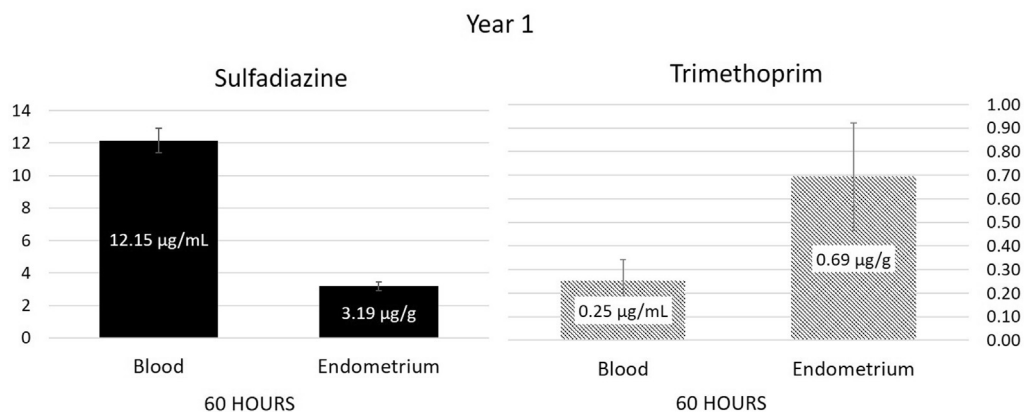


Fig. 1. Concentrations of sulfadiazine ($n = 21$) and trimethoprim ($n = 4$) in peripheral blood and endometrium at 60 h after beginning treatment (mean \pm standard error of the mean). Correlation between blood and endometrium for sulfadiazine was $r = 0.898$ and that for trimethoprim was $r = 0.936$ in year 1 ($P < .001$).

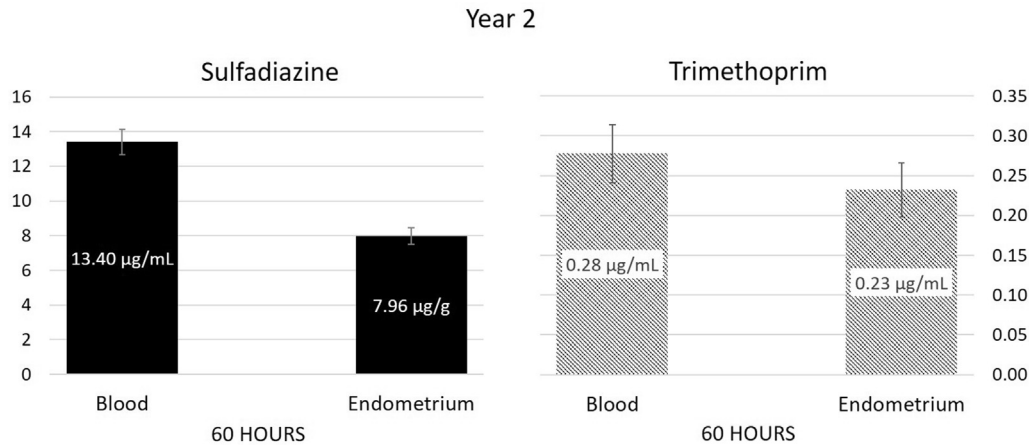


Fig. 3. Concentrations of sulfadiazine ($n = 20$) and trimethoprim ($n = 20$) in peripheral blood and endometrium at 60 h after beginning treatment (mean \pm standard error of the mean) and correlation between blood and endometrium in year 2 ($P < .001$). Correlations between blood and endometrial sulfadiazine and trimethoprim were $r = 0.819$ and $r = 0.948$, respectively ($P < .0001$).

drug distribution within endometrial tissue encourages follow-up clinical studies.

A recurrent concern when using systemic antibiotics, and often a reason to choose intrauterine options, is the risk of adverse effects on gastrointestinal flora [6]. Even though our study was not designed to assess these endpoints, there were no obvious effects on gastrointestinal function noticed during or after treatment. This agrees with findings, by others, of a lack of major changes in the intestinal flora of horses receiving a 5-day treatment of either oral or intravenous sulfadiazine-trimethoprim [22]. There is currently widespread use of human-labeled sulfamethoxazole-trimethoprim tablets in equine hospitals and horse farms. It is important to mention that while sulfadiazine-trimethoprim combination investigated in the present study is approved by the Food and Drug Administration (FDA) for use in equine species, sulfamethoxazole in combination with trimethoprim is not, and safety studies are lacking.

In conclusion, the sulfadiazine-trimethoprim formulation investigated could represent a cost-effective option for systemic treatment of bacterial endometritis. Our study provides data to warrant further investigation to ultimately show the clinical efficacy of this FDA-approved oral suspension in the treatment of bacterial endometritis in the mare.

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