Application of in vivo microdialysis for investigation of unbound drug concentrations of intravenously administered sulfadimidine in the paranasal sinus mucosa of horses

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OBJECTIVE
To monitor concentrations of sulfadimidine in the paranasal sinus mucosa (PSM) of unsedated horses following IV administration of trimethoprim-sulfadimidine via in vivo microdialysis.

ANIMALS
10 healthy adult horses.

PROCEDURES
Concentric microdialysis probes were implanted into the subepithelial layers of the frontal sinus mucosa of standing sedated horses. Four hours after implantation, trimethoprim-sulfadimidine (30 mg/kg) was administered IV every 24 hours for 2 days; dialysate and plasma samples were collected at intervals during that 48-hour period and analyzed for concentrations of sulfadimidine. The dialysate concentration and relative loss of sulfadimidine from the perfusate were used to calculate the PSM concentration.

RESULTS
Microdialysis probe implantation and subsequent in vivo microdialysis were successfully performed for all 10 horses. Following the first and second administration of trimethoprim-sulfadimidine, mean ± SD peak concentrations of sulfadimidine were 55.3 ± 10.3 μg/mL and 51.5 ± 8.7 μg/mL, respectively, in plasma and 9.6 ± 4.5 μg/mL and 7.0 ± 3.3 μg/mL, respectively, in the PSM. Peak sulfadimidine concentrations in the PSM were detected at 5.9 ± 2.7 hours and 5.4 ± 2.3 hours following the first and second drug administrations, respectively. For 12 hours, mean PSM sulfadimidine concentration remained greater than the minimum inhibitory concentration indicative of sulfonamide susceptibility of equine bacterial isolates (4.75 μg/mL).

CONCLUSIONS AND CLINICAL RELEVANCE
In vivo microdialysis for continuous monitoring of PSM sulfadimidine concentrations in unsedated horses was feasible. Intravenous administration of trimethoprim (5 mg/kg) and sulfadimidine (25 mg/kg) proved likely to be efficient for treating sinusitis caused by highly susceptible pathogens, providing that the dosing interval is 12 hours. (*Am J Vet Res* 2015;76:318–327)

In horses, sinusitis is the most frequent disorder of the paranasal sinuses.[1] Possible causes include infections of the upper respiratory tract (primary sinusitis), dental disease, facial trauma, sinus cysts, progressive ethmoid hematoma, sinonasal mycosis, or neoplasia (secondary sinusitis).[2,3] The clinical seriousness of equine sinonasal disorders derives from their chronicity and poor response to treatment.[3,4] Anti-infective drugs are frequently used in treatment of equine sinusitis, although lack of long-term clinical cure has been reported repeatedly.[5–8] It has been suggested that failure of anti-infective treatment is caused by accumulation of inspissated pus within individual sinus compartments in horses with primary sinusitis and by persistence of underlying conditions in horses with secondary sinusitis.[6,9,10] However, given that treatment failure of anti-infective agents in general might occur because of subinhibitory concentrations within the infected tissue,[11,12] this aspect should be considered when medicating equids with sinusitis.

Trimethoprim-sulfonamide combinations are frequently used in equine medicine and are considered appropriate for treatment of infections of the equine respiratory tract.[13,14] Following administration of such drug combinations to horses, trimethoprim and sulfonamide concentrations have been detected in various body fluids and tissues including blood, urine, synovia, endometrium, CSF, peritoneal fluid,[15,16] bron-

ABBREVIATIONS

AUC	Area under the curve
Cmax	Maximum plasma concentration
HPLC	High-performance liquid chromatography
ISF	Interstitial fluid
MIC	Minimum inhibitory concentration
PSM	Paranasal sinus mucosa

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chial epithelial lining fluid,[17] and subcutaneous tissue chamber fluid.[18,19] However, there are apparently no data available regarding drug concentrations of either trimethoprim or sulfonamides within the paranasal sinuses of horses.

Drug concentrations can vary considerably among different tissues. It has therefore been recommended not to extrapolate target tissue concentrations of anti-infective agents from data determined for the plasma compartment or a different peripheral tissue.[11,20] Moreover, the clinical outcome of an anti-infective treatment closely correlates with achievement and maintenance of effective drug concentrations at the site of infection, which is the ISF in most cases of bacterial infections.[21] Therefore, drug concentrations of anti-infective agents should be obtained not only from the target tissue, but also directly from the ISF of the tissue of interest.[11,22]

Microdialysis is considered a suitable technique for continuous monitoring of endogenous and exogenous compounds in body fluids and in the ISF of tissues in humans and other animals.[20,22–24] However, it has apparently not been used in the paranasal sinuses of any species. Several reports[25–27] have addressed the principles of microdialysis. In brief, the tip of a microdialysis probe (containing a semipermeable membrane) is introduced into a tissue or body fluid. The probe is perfused with a perfusion solution, and an exchange of substances from the perfusion solution into the periprobe fluid and vice versa takes place. The bidirectional mass transport across the membrane pores is driven by a concentration gradient. Analysis of the resulting sample (dialysate) indicates gain or loss of substances to or from the perfusion solution. The term recovery has been applied to describe the relation of substances in the periprobe fluid with those in the dialysate.[25]

The objective of the study reported here was to develop and evaluate a technique that allows continuous monitoring of anti-infective drug concentrations within the PSM of unsedated horses. A specific goal was to determine concentrations of sulfadimidine in the PSM of horses following IV administration of trimethoprim-sulfadimidine. We hypothesized that in vivo microdialysis would be a suitable technique to quantify concentrations of sulfadimidine within the PSM of horses following IV administration of the drug.

Materials and Methods

Horses

Ten university-owned adult horses of various breeds (9 mares and 1 stallion) were used. Horses weighed from 410 to 675 kg (mean, 558.2 kg) and ranged in age from 5 to 23 years (mean, 13.5 years). All horses were considered healthy on the basis of results of a general physical examination, routine hematologic evaluation, and serum biochemical analysis. None of the horses had a history of or current clinical signs of paranasal sinus disease. For each horse, lateral and dorsoventral radiographic views of the paranasal sinuses revealed no abnormalities. Horses had not received any medical treatment for at least 3 weeks before study enrollment. Horses were housed in individual stalls, were fed hay and mixed feed twice daily, and had ad libitum access to water. All procedures were approved by the Ethical Committee of the Lower Saxony State Office for Consumer Protection and Food Safety.

Microdialysis equipment and probe implantation

Highly flexible, concentric microdialysis probes[a] **(Figure 1)** were used in the study. Prior to in vivo implantation, every probe was tested under in vitro conditions to verify probe integrity and performance variables (data not shown). After in vitro testing, probes were stored in double-distilled water. Before in vivo application, probes were flushed with sterile-filtered PBS solution (pH, 7.4) and kept in PBS solution until immediately prior to implantation.

The implantation of the microdialysis probe was performed in standing horses, each of which was sedated with detomidine hydrochloride (0.015 to 0.03 mg/kg) and butorphanol tartrate (0.1 mg/kg) administered IV. For each horse, a 12-gauge indwelling IV catheter was placed in a jugular vein with aseptic technique. The dorsolateral aspect of the head was prepared for aseptic surgery, and the proposed incision lines were infiltrated SC with 2% lidocaine hydrochloride. Access to the mucosa of the frontal sinus was achieved by drilling an opening (1 to 2 mm in diameter) into the frontal bone without perforation of the underlying sinus mucosa (endoscopic guidance provided via the caudal maxillary sinus). Subsequently, a fluid-filled blister (sterile-filtered PBS solution; diameter, 20 to 30 mm) was created within the subepithelial layers of the mucosa. To achieve this, the Luer tip of a 20-mL syringe, filled with PBS solution, was positioned on the depression, and light pressure was applied. The tip of a microdialysis probe (including the semipermeable membrane; length, 10 mm; outer diameter, 0.6 mm) was placed flat under the bone in the blister, and the complete microdialysis system was affixed to the horse (Figure 1). The shaft of the probe was secured to the skin of the horse's forehead with tissue glue. The outlet tube (length, 220 mm; outer diameter, 1 mm) with the vial holder was taped to the horse's forelock, whereas the inlet tube (length, 600 mm; outer diameter, 1 mm) was connected to a microdialysis syringe.[b] The latter was placed in a battery-powered microdialysis pump,[c] which in turn was secured in a lightweight metal box taped to the horse's halter on its neck. The microdialysis probe was removed after a total sample collection time of 52 hours beginning 4 hours after the implantation. The skin incisions were closed with disposable skin staples or simple interrupted stitches.

Postsurgical treatment

After microdialysis probe implantation, each horse underwent a routine physical examination once

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daily for 2 weeks or until euthanasia because of enrollment in an unrelated study (5 to 28 days after probe implantation; n = 6). Total protein concentration and PCV were determined on the day of probe implantation and on the following 2 days. Staples or stitches were removed 14 days after surgery, and the cosmetic outcome for the 4 remaining horses was assessed 30, 60, and 90 days after surgery.

Histologic analysis

Mucosa samples from the central area of the blister including the microdialysis probe and the underlying frontal bone were obtained from one of the study horses that was euthanized (because of enrollment in an unrelated study) 5 days after microdialysis probe implantation and subsequent performance of in vivo microdialysis. Tissue sections were stained with toluidine blue and examined by light microscopy.

In vivo microdialysis

At the beginning of each in vivo experiment, each horse's microdialysis probe was subjected to an in vivo calibration that was subdivided into 3 successive phases (adapted from a method published by Bouw and Hammarlund-Udenaes[28]). The probe was perfused with PBS solution (duration, 90 minutes; flow rate, 2 µL/min) to obtain a blank sample. Then, the probe was perfused with PBS solution containing sulfadimidine (1 µg/mL) and sulfadiazine (internal standard; 2 µL/min) for a period of 90 minutes (reference period). After that, a washout period of 60 minutes was allowed to ensure removal of sulfadimidine from the microdialysis probe and the periprobe fluid. During the washout period and the following experimental period, the probe was continuously perfused with PBS solution containing sulfadiazine (1 µg/mL) at a flow rate of 2 µL/min. The total time for the in vivo calibration was 240 minutes.

On completion of in vivo calibration, the experimental period commenced. Each horse was given a trimethoprim-sulfadimidine formulation[d] (30 mg/kg, IV) twice. Administration of the first dose (0 hours) defined the start of the 48-hour experimental period; the second dose was administered 24 hours later. During the experimental period, dialysate fractions were collected at 90-minute intervals. The volume of each dialysate fraction was measured, and the maximum available aliquot was transferred from the microvial to a glass vial and diluted to 290 µL with PBS solution. Samples were then frozen and kept at 20°C until analysis. Blood samples (10 mL) were collected from the jugular vein catheter into EDTA-containing tubes prior to and at predetermined intervals (0.5, 1, 1.5, 3, 6, 12, 18, and 24 hours) after each dose administration. The 24-hour blood and dialysate samples obtained after the first dose administration were collected immediately prior to administration of the second dose. Blood samples were centrifuged at 900 X *g* for 6 minutes; plasma was then transferred to plastic tubes and frozen at –20°C until analysis.

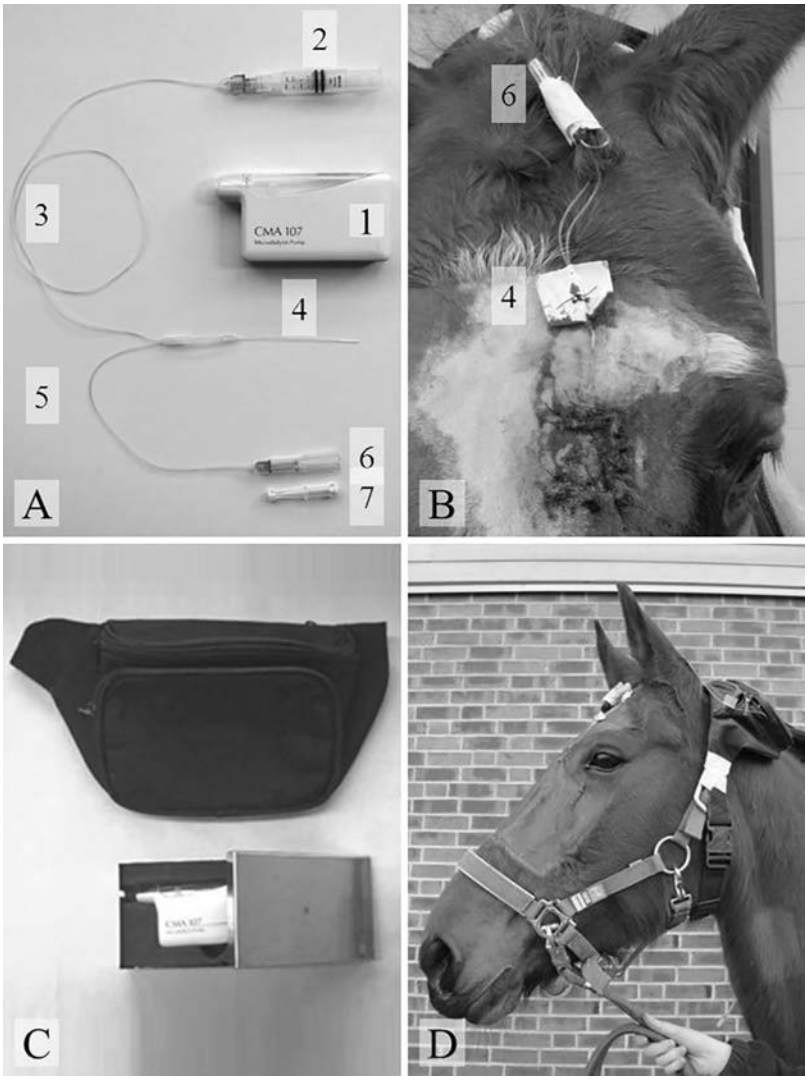

Figure 1—Photographs of the microdialysis system and its fixation on a horse, as used in a study to develop and evaluate a technique that allows continuous monitoring of sulfadimidine concentration in the PSM of horses following IV administration of trimethoprim-sulfadimidine. A—The applied microdialysis system consists of a microdialysis pump (1), microdialysis syringe (2), microdialysis probe (inlet tubing [3], probe shaft [4], outlet tubing [5], vial holder [6]), and microvial (7). B—The microdialysis probe shaft (4) is affixed to the forehead of a horse, with the vial holder (6) attached to the forelock (bottom side up). The horse is sedated during implantation of the probe in the frontal sinus mucosa; however, sedation is not required during the period of microdialysis. C—The microdialysis pump is placed inside a metal box and then into a bag. D—The bag is positioned on the horse's neck and taped to the halter.

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Drug analysis

The thawed dialysate fractions directly underwent HPLC, whereas plasma samples underwent an extraction procedure adapted from Nouws et al[29] prior to drug analysis. An aliquot (500 µL) of each plasma sample was transferred to an extraction tube and spiked with the internal standard, sulfadiazine (10 µg/mL). Then, 1,000 µL of perchloric acid (0.66 mol/L) was added to each sample. After mixing for 10 seconds, 12 µL of saturated K_2CO_3 solution and 10 µL of 4% NaCl solution were added. The tubes were placed inside a vibrating shaker for 30 minutes. Subsequently, samples were centrifuged at 4,500 X *g* for 10 minutes. An aliquot of 300 µL of each resulting supernatant was transferred into a HPLC glass vial, whereas the residue was dissolved in 1,000 µL of perchloric acid and processed for a second time. The HPLC samples were stored at –20°C until analysis. Following HPLC, results for the 2 extraction stages of each plasma sample were summed.

All test compounds were quantified by use of an HPLC system,[e] with associated software,[f] by the external standard method. An HPLC cartridge[g] (5 µm) and guard column[h] (5 µm) were placed in an HPLC column heater[i] at 40°C. The eluent consisted of 15% methanol (HPLC grade) and 85% McIlvaine citrate buffer (pH, 2.2; 20.8 g of citric acid and 0.4 g of $Na_2HPO_4 \cdot H_2O$ in 1 L of purified water). Aliquots of 100 µL were injected and analyzed at 254 nm (flow rate, 1.0 mL/min). Linear standard curves of sulfadimidine and sulfadiazine ranged from 0.07 (sulfadimidine) and 0.08 µg/mL (sulfadiazine) to 10 µg/mL (spiked dialysate and plasma samples). The limit of detection was 0.07 µg/mL for sulfadimidine and 0.08 µg/mL for sulfadiazine. The limit of quantification was 0.14 µg/mL for sulfadimidine and 0.15 µg/mL for sulfadiazine. The mean ± SD recovery of sulfadiazine from plasma samples was 74.1 ± 11.9%.

Calculation of recovery rates and PSM concentrations of sulfadimidine and sulfadiazine

Recovery rates of sulfadimidine and sulfadiazine were assessed according to the retrodialysis method.[29] Thus, relative drug loss from the perfusion solution into the periprobe fluid was determined for the reference period (sulfadimidine and sulfadiazine) and the experimental period (sulfadiazine) as follows:

$$\text{Relative loss} = (C_P - C_D)/C_P$$

where C_P is the concentration of sulfadimidine or sulfadiazine in the perfusion solution and C_D is the concentration of either sulfadimidine or sulfadiazine in the dialysate.

The relative loss of sulfadimidine during the reference period was used to estimate unbound sulfadimidine concentrations in the periprobe fluid during the experimental period (retrodialysis by drug). The relative loss of sulfadiazine during the experimental period was calculated to evaluate potential alterations of recovery rates for the remainder of the experiment (retrodialysis by calibrator). In the present study, the periprobe fluid was the content of the blister within the subepithelial layers of the PSM. To determine the corrected dialysate fraction of unbound sulfadimidine within the PSM, the sulfadimidine concentration of each dialysate fraction during the experimental period was divided by the relative loss of sulfadimidine during the reference period.

Data analysis

Pharmacokinetic parameters of sulfadimidine in plasma and the PSM were determined for each horse by noncompartmental analysis.[j] The data obtained included Cmax, time of Cmax, drug concentration at the last observation of the respective dosing interval, elimination half-life, elimination rate constant, AUC, area under the first moment curve, mean residence time, volume of distribution at steady state, and total plasma clearance. Values are reported as mean ± SD.

Results

Implantation of the microdialysis probe was successful in all 10 horses. Histologic examination of tissue samples obtained from 1 horse that was euthanized (because of enrollment in an unrelated study) 5 days after microdialysis probe implantation and subsequent performance of in vivo microdialysis verified the position of the microdialysis probe within the surgically created blister and thus within the subepithelial layers of the PSM. Subsequent performance of in vivo microdialysis was successful for a period of 52 hours for all horses.

Horses tolerated the processes of probe implantation and in vivo microdialysis very well. Total protein concentration and PCV were within reference limits in all horses until the end of the in vivo microdialysis procedures. For all horses, pulse rate, respiratory rate, and rectal temperature remained within reference limits up to 14 days after surgery or until euthanasia. Short- and long-term outcome of the surgical procedure were very good. In 4 horses with long-term follow-up (≥ 3 months), all incisions healed by first intention. The hair regrew and scars were barely detectable; cosmetic results at day 90 were deemed excellent.

For the 10 horses, plasma samples contained mean ± SD peak concentrations of sulfadimidine of 55.3 ± 10.3 µg/mL and 51.5 ± 8.7 µg/mL following the first and second treatment with trimethoprim-sulfadimidine, respectively **(Figure 2)**. Pharmacokinetic parameters for sulfadimidine in plasma were summarized **(Table 1)**.

The performance of the microdialysis system was fully satisfactory, and the amounts of dialysate collected at each time point were consistent. The mean volume of the dialysate fractions available for analytic purposes was 122.7 ± 26.8 µL/sample.

Results for relative loss of sulfadimidine during the reference period were available for 8 of 10 horses (mean, 45.9 ± 8.8%; **Table 2**). Reference samples from

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2 horses were not evaluable owing to analytic difficulties. Therefore, the mean relative loss of sulfadimidine of the other 8 horses was applied for these 2 horses.

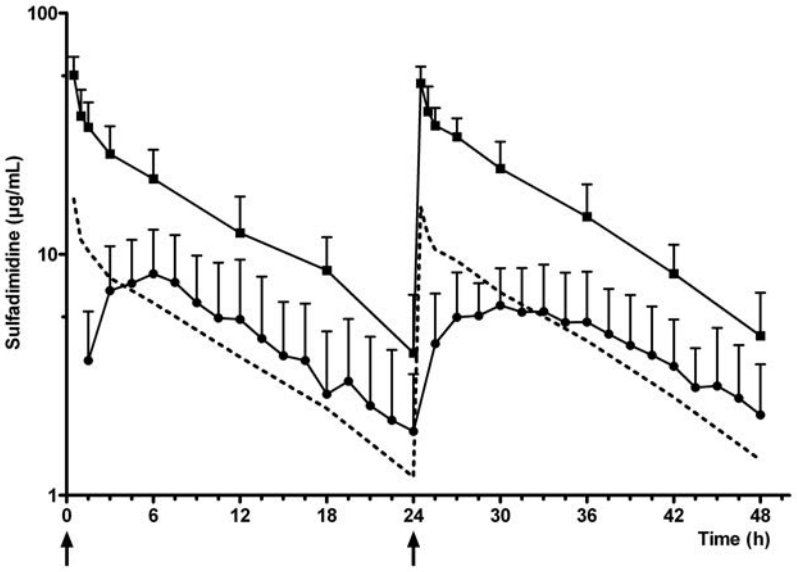

Figure 2—Concentration-time profiles of total plasma (squares), theoretical unbound plasma (dotted line), and PSM (circles) sulfadimidine concentrations in 10 horses (each equipped with the microdialysis system in Figure 1) following IV administration of trimethoprim-sulfadimidine (30 mg/kg [25 mg of sulfadimidine/kg], IV) at 0 and 24 hours (arrows). Blood samples were collected at predetermined intervals (0.5, 1, 1.5, 3, 6, 12, 18, and 24 hours) after each dose administration. Sulfadimidine concentrations in PSM were estimated from assessments of dialysate samples collected at 90-minute intervals after each dose administration in relation to the relative loss of sulfadimidine from the perfusate. The 24-hour blood and dialysate samples obtained after the first dose administration were collected immediately prior to administration of the second dose. Data are reported as mean ± SD.

Corrected dialysate fractions yielded detectable concentrations of sulfadimidine throughout the experiment **(Figure 3)**; the mean peak concentrations were 3.8 ± 2.0 µg/mL following the first dose of trimethoprim-sulfadimidine and 2.7 ± 1.0 µg/mL following the second drug dose. To provide temporally averaged estimates of the PSM concentration for the respective sample interval, corrected dialysate fractions and relative loss of sulfadimidine from the perfusate were analyzed. The mean peak PSM concentrations of sulfadimidine were 9.6 ± 4.5 µg/mL and 7.0 ± 3.3 µg/mL following the first and second drug dose, respectively. Substantial differences were evident in the PSM concentration of sulfadimidine among individual horses, exemplified by peak concentrations ranging from 2.7 to 15.3 µg/mL pharmacokinetic parameters for sulfadimidine in the PSM were summarized (Table 1).

Results for relative loss of sulfadiazine were available for 7 of 10 horses, with a mean of 40.0 ± 17.7% during the reference period and 22.0 ± 11.9% during the experimental period. For 3 horses, calculations of relative loss of sulfadiazine revealed no loss, but gain of sulfadiazine in reference samples as well as only minimal loss or gain of sulfadiazine in most of the dialysate frac-

Table 1—Pharmacokinetic parameters of sulfadimidine in plasma samples and the PSM determined for 10 horses (each equipped with the microdialysis system) during a 48-hour period following IV administration of trimethoprim-sulfadimidine (30 mg/kg [25 mg of sulfadimidine/kg], IV) at 0 and 24 hours.

	Plasma		PSM	
Parameter	**0–24 hours**	**24–48 hours**	**0–24 hours**	**24–48 hours**
Cmax (µg/mL)	55.3 ± 10.5	51.5 ± 8.7	9.6 ± 4.5	7.0 ± 3.3
Drug concentration at the last observation of the respective dosing interval (µg/mL)	5.0 ± 2.6	4.6 ± 2.3	1.9 ± 1.2	2.2 ± 1.3
Tmax (h)	—	—	5.9 ± 2.7	5.4 ± 2.3
Elimination half-life (h)	7.7 ± 2.0	7.7 ± 1.5	8.4 ± 2.5	10.9 ± 4.4
Elimination rate constant (h^{-1})	0.10 ± 0.03	0.09 ± 0.02	0.08 ± 0.04	0.07 ± 0.04
AUC (µg/mL•h)	373.2 ± 105.6	419.4 ± 101.5	110.7 ± 56.7	103.8 ± 51.2
Area under the first moment curve (µg/mL•h^2)	2,726.3 ± 1,185.2	3,163.0 ± 1,022.0	1,126.6 ± 701.7	1,129.8 ± 613.6
Mean residence time (h)	7.0 ± 1.4	7.4 ± 0.7	10.0 ± 0.7	10.8 ± 1.1
Clearance (mL/h/kg)	63.6 ± 21.9	56.6 ± 17.7	—	—
Volume of distribution at steady state (mL/kg)	633.2 ± 160.3	567.3 ± 72.9	—	—

Each horse was instrumented with a microdialysis system. Access to the mucosa of the frontal sinus was achieved by drilling a 1- to 2-mm-diameter opening in the frontal bone without perforation of the underlying sinus mucosa. Subsequently, a fluid-filled blister (sterile-filtered PBS solution; diameter, 20 to 30 mm) was created within the subepithelial layers of the mucosa. The tip of a microdialysis probe (including the semipermeable membrane) was placed flat under the bone in the blister. Blood samples were collected at 0.5, 1, 1.5, 3, 6, 12, 18, and 24 hours after each dose administration. Dialysate samples from the blister were collected at 90-minute intervals after each dose administration. The 24-hour blood and dialysate samples obtained after the first dose administration were collected immediately prior to administration of the second dose. The relative loss of sulfadimidine during a 90-minute reference period was used to estimate unbound sulfadimidine concentrations in the periprobe fluid (ie, PSM) during the experimental period (retrodialysis by drug). To determine the corrected dialysate fraction of unbound sulfadimidine within the PSM, the sulfadimidine concentration of each dialysate fraction during the experimental period was divided by the relative loss of sulfadimidine during the reference period.

Tmax = Time of Cmax. — = Not applicable.

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Table 2—Comparison of in vivo retrodialysis recoveries of sulfadimidine and sulfadiazine for the 10 individual horses (each equipped with the microdialysis system) in Table 1.

	Reference period (1 sample/horse)		Experimental period 3 (2 samples/horse)
Horse	Relative loss of sulfadimidine	Relative loss of sulfadiazine	Relative loss of sulfadiazine
1	—	—	—
2	44.9	–2.1*	–14.9 ± 28.9*
3	47.3	23.7	14.2 ± 5.7
4	34.4	73.0	43.5 ± 20.7
5	35.9	–6.9*	1.6 ± 9.7*
6	—	35.4	17.3 ± 12.8
7	45.7	18.3	9.6 ± 8.9
8	43.5	40.3	27.8 ± 7.9
9	54.0	44.2	17.6 ± 9.8
10	61.2	44.8	24.2 ± 17.6
Mean ± SD	45.9 ± 8.8	40.0 ± 17.7	22.0 ± 11.9

At the beginning of each in vivo experiment, each horse's microdialysis probe was calibrated in 3 successive phases. The probe was perfused with PBS solution (duration, 90 minutes; flow rate, 2 µL/min) to obtain a blank sample. Then the probe was perfused with PBS solution containing sulfadimidine (1 µg/mL) and sulfadiazine (internal standard; 2 µL/min) for a period of 90 minutes (reference period). After that, a washout period of 60 minutes was allowed to ensure removal of sulfadimidine from the microdialysis probe and the periprobe fluid. During the washout period and the following experimental period, the probe was continuously perfused with PBS solution containing sulfadiazine (1 µg/mL) at a flow rate of 2 µL/min. On completion of in vivo calibration, the experimental period commenced. Relative drug loss from the perfusion solution to the periprobe fluid was determined for the reference period (sulfadimidine and sulfadiazine) and the experimental period (sulfadiazine) as follows: Relative loss = $(C_P - C_D)/C_P$, where C_P is the concentration of sulfadimidine or sulfadiazine in the perfusion solution and C_D is the concentration of either sulfadimidine or sulfadiazine in the dialysate. Relative loss of sulfadimidine for horses 1 and 6 and relative loss of sulfadiazine during the reference and experimental periods for horse 1 were excluded because of analytic difficulties.

*Relative losses of sulfadiazine during the reference and experimental periods for horses 2 and 5 revealed no or minimal loss or gain of sulfadiazine and thus were excluded from further calculations.

— = Not available.

tions during the experimental period (Table 2). Values for these 3 horses were excluded from further calculations. In all horses, considerable variations in relative loss of sulfadiazine (experimental period) were evident as well as a mild negative change in relative loss over the experimental duration (48 hours); considering the mean of the first and the last 5 dialysate fractions of the experimental period (7/10 horses), relative loss of sulfadiazine decreased by 24%.

Discussion

To the authors' knowledge, this is the first study to investigate the application of the microdialysis technique in the PSM of unsedated horses. Furthermore, following IV administration of trimethoprim-sulfadimidine, concentrations of sulfadimidine were detected within the PSM in horses by means of continuous in vivo microdialysis.

Probe implantation was successfully performed in all 10 study horses. The procedure itself was tolerated well by all horses. However, excellent sedation

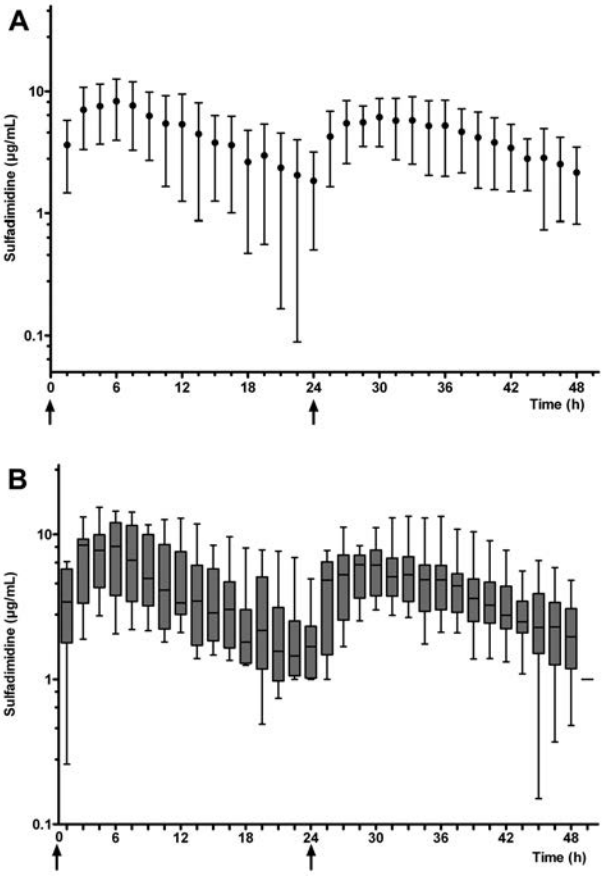

Figure 3—Concentrations of sulfadimidine in the PSM of 10 horses (each equipped with the microdialysis system in Figure 1) estimated from assessments of dialysate samples collected at 90-minute intervals in relation to the relative loss of sulfadimidine from the perfusate following IV administration of trimethoprim-sulfadimidine (30 mg/kg [25 mg of sulfadimidine/kg], IV) at 0 and 24 hours (arrows). A—Plot of mean ± SD PSM concentration of sulfadimidine. B—Box-and-whisker plots of PSM concentrations of sulfadimidine to illustrate data variation. For each box, the horizontal line represents the median, and the upper and lower boundaries represent the 75th and 25th percentiles, respectively. Whiskers represent the minimum and maximum. The MICs for sulfonamides in combination with trimethoprim recommended as a guideline for antimicrobial susceptibility of bacterial organisms isolated from horses are 4.75 µg of sulfadiazine/mL or 9.5 µg of sulfadiazine or sulfamethoxazole/mL.

was required during the process of probe implantation, especially during probe insertion, because even minor movements of the horse's head could damage the probe, the mucous membrane, or both. The limiting factor for probe implantation was successful creation of a fluid-filled blister within the subepithelial soft connective tissue of the PSM. Still, there was an obvious learning curve to probe implantation; successive procedures resulted in a more easily performed probe implantation process.

Horses tolerated performance of the in vivo microdialysis experiment over the entire 52-hour period very well. However, selection of docile, well-mannered horses is recommended, given that repeat-

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ed handling of the microdialysis system requires increased interaction with the horses for the duration of the experiment. The excellent healing and cosmetic outcomes for the horses in the present study might be a consequence of the exclusive enrollment of horses without disease of the paranasal cavities or application of trimethoprim-sulfadimidine at a therapeutic dosage (30 mg/kg, IV, q 24 h).

Potentiated sulfonamides are frequently used in equine medicine (eg, in treatment of respiratory tract infections).[13,14] Unlike trimethoprim, which has a short half-life and low tissue concentrations in horses[30] and would thus require considerable analytic effort to measure accurately in dialysate samples because of expected low concentrations, sulfadimidine in dialysate samples was readily detected by HPLC. Following IV injection of trimethoprim-sulfadimidine, concentrations of sulfadimidine were detected both in plasma and in dialysate fractions (Figure 2). The concentrations and pharmacokinetic parameters of sulfadimidine in plasma samples collected from horses in the present study were consistent with previously reported data[17,31,32] for sulfonamides in horses.

In the present study, peak sulfadimidine concentrations in the PSM, estimated from the dialysate fraction data, were approximately 8 µg/mL. These results are in accordance with those of previous studies[15–17,33] that investigated concentrations of sulfonamides within various tissues and body fluids including synovia, peritoneal fluid, pulmonary epithelial lining fluid, and endometrium. By use of dosing regimens comparable to that used in the present study, peak drug concentrations ranged from 5 to 25 µg/mL, depending on the investigated tissue or body fluid. van Duijkeren et al[18] and Ensink et al[19] investigated the distribution of sulfadiazine and trimethoprim into noninfected or infected subcutaneous tissue chamber fluid of ponies and reported sulfadiazine concentrations of approximately 20 µg/mL in the tissue chamber fluid following IV or oral administration of sulfadiazine (25 mg/kg).

Plasma protein binding of sulfadimidine in horses has been reported as 69.4 ± 2.5%.[29] In the present study, the AUC of unbound sulfadimidine in the PSM (ie, periprobe fluid) was 29.7% (from 0 to 24 hours) and 24.8% (from 24 to 48 hours) of the AUC of total plasma sulfadimidine. Considering that only unbound drug fractions are able to penetrate uninflamed biological barriers, it is not surprising that the concentration-time profile of sulfadimidine in the PSM closely resembled the theoretical concentration of the unbound sulfadimidine fraction in plasma following achievement of maximum tissue concentrations approximately 6 hours after systemic drug administration (Figure 2). The free fraction of an anti-infective agent passes from plasma to the tissue compartment and has thus been previously described as a valuable substitute for tissue concentrations.[34] Results of the present study indicated that after IV drug application, the free plasma fraction allowed precise predictions of tissue concentrations, but only after attainment of maximum tissue concentrations. The present study revealed that in vivo microdialysis provided continuous measurements of anti-infective agents within the equine PSM, which is considered the site of infection in equine sinusitis, during each entire dosing interval. This is of particular importance because bacterial pathogens often reside in the ISF and knowledge of the concentration of antimicrobials achieved in this compartment would assist in predicting the drugs' clinical efficacy in treatment of soft tissue infections. Reliance on the plasma concentration alone would result in overestimation of the efficacy of trimethoprim-sulfadiazine formulations.[18]

The estimated PSM concentrations were evaluated relative to MICs of potentiated sulfonamides. Minimum inhibitory concentrations of ≤ 0.25 µg of trimethoprim/mL and 4.75 µg of sulfadiazine/mL have been recommended as a guideline for antimicrobial effectiveness against susceptible bacterial organisms isolated from horses.[35] The same MICs were determined for trimethoprim-sulfamethoxazole in a 1:19 ratio against bacteria involved in equine lower airway infections (*Streptococcus equi* subsp *zooepidemicus* and *Staphylococcus aureus*).[17] In the present study, the mean PSM sulfadimidine concentration exceeded the recommended MIC for approximately 12 hours after IV administration of trimethoprim-sulfadimidine (30 mg/kg). However, because of the large variations of Cmax among individual horses, sulfadimidine concentrations only exceeded the designated MIC for 12 hours in the PSM of 5 of 10 horses. Other authors[36] have recommended MICs of ≤ 0.5 µg of trimethoprim/mL and 9.5 µg of sulfadiazine or sulfamethoxazole/mL for equine bacterial isolates; in the present study, mean peak concentrations of sulfadimidine exceeded that latter MIC only temporarily in 5 of 10 horses. The PSM sulfadimidine concentrations did not attain values higher than the MICs reported by Ensink et al[37] (1 µg of trimethoprim/mL and 20 µg of sulfadiazine or sulfamethoxazole/mL) and Fey and Schmid[38] (0.5 µg of trimethoprim/mL and 32 µg of sulfadiazine or sulfamethoxazole/mL). However, on the basis of mean sulfadimidine concentrations within the PSM and assuming effective trimethoprim concentrations, we concluded that IV treatment with sulfadimidine (25 mg/kg) in combination with trimethoprim (5 mg/kg) was likely to be efficient for treating sinusitis caused by pathogens that were highly susceptible to those drugs in horses. Nevertheless, the dosing interval should be adapted to 12 hours to ensure effective drug concentrations throughout the entire period between doses. Similar observations have previously been made with regard to infections of the lower respiratory tract in horses.[17]

In the present study, sulfadimidine concentrations in the PSM varied considerably among individual horses, exemplified by peak concentrations ranging from 2.7 to 15.3 µg/mL. To interpret these differences, it is important to take into account not only biological reasons but also potential sources of error due to technical issues.

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Microdialysis is considered to be a semi-invasive technique with minimal impact on affected tissue.[39] Tissue trauma is usually caused by probe implantation, rather than by the probe itself.[25,27] In the present study, probe implantation required surgical access to each horse's frontal sinus mucosa. Moreover, creation of a fluid-filled blister within the subepithelial layers of the sinus mucosa was required to enable insertion of the microdialysis probe (outer diameter of membrane area, 600 µm) into the subepithelial layers of the frontal sinus mucosa, which measures only 110 to 120 µm in horses.[k] Accordingly, the microdialysis probe was surrounded not by naturally occurring ISF or other body fluids, as is typical for in vivo microdialysis,[25] but by the content of the sinus blister. Consequently, the estimated drug concentration within the PSM blister may have varied from concentrations in unaffected ISF for several reasons. First, blister creation resulted in a dilution that might have reduced drug concentration within the blister. As the blister size could be neither measured nor influenced during blister creation, the dilution factor was unknown and may have varied among individual horses. Second, blister creation provided an altered ratio of the capillary surface area to the volume of the tissue and may have influenced drug delivery into the PSM.[40] Furthermore, separation of the well-vascularized subepithelial layers of the PSM[4] likely led to rupture of arterioles and venules and subsequent minor hemorrhage within the blister. Leakage of intercellular fluid in the blister may have resulted in drug dilution. Although hemorrhage was apparently limited by coagulation, it cannot be excluded that drug delivery to the PSM was altered by microbleeding and temporary alterations in capillary permeability.

The major issue for making accurate estimations of true tissue concentrations from microdialysate samples is the reliability of the applied recovery method.[23] In the present study, recovery rates of sulfadimidine were estimated by a technique of retrodialysis by drug because the substance of interest is considered the ideal calibrator in microdialysis experiments.[28] Recovery rates of sulfadimidine during the reference period were constant in 8 horses (45.9 ± 8.8%). Consequently, the mean relative loss of sulfadimidine was used as a measure of recovery in the 2 horses for which the actual recovery rate of the particular probe was not available because of analytic issues.

However, retrodialysis by drug needs to be performed prior to the actual in vivo experiment and cannot take into account changes of recovery rates over time. Therefore, additional application of an internal standard is recommended to observe changes in the recovery rate during the entire in vivo experiment.[25] In the present study, sulfadiazine was used as an internal standard because it appeared appropriate in preliminary in vitro experiments. Some difficulties were encountered during the in vivo application of sulfadiazine. First, calculations of relative loss of sulfadiazine revealed only minimal loss or even gain of sulfadiazine in dialysate fractions of 3 horses. Although the reasons for this observation were not apparent, we assumed bioanalytical issues were the cause given the low drug concentrations in all solutions and samples. This effect might have been increased by the necessity of diluting the dialysate samples for HPLC analysis. Second, in vivo results of relative loss of sulfadiazine had considerable fluctuation in values obtained during the experimental period in single probes (SD up to 20.7%; Table 2). However, these fluctuations were not evident in the concentration-time profile of sulfadimidine. Bouw and Hammarlund-Udenaes[28] observed similar discrepancies and explained them with the incidence of low retrodialysis recoveries in general. Third, considerable variations between relative loss of sulfadiazine from the reference period (40%) and the experimental period (22%) were evident. Furthermore, a reduction of relative loss of sulfadiazine of 24% was observed during the experimental period. This reduction of recovery rates may have been a result of progressive occlusion of the probe membrane pores.[25] In addition, it is possible that microbleeding (caused by blister creation) was still present at the beginning of the in vivo experiment and caused an initial increase of recovery rates. Both of these circumstances would also affect recovery rates of sulfadimidine; consequently, final sulfadimidine concentrations would be higher than estimated. Because of invasive surgical interventions in the present study and to prevent alterations of recovery rates and estimated drug concentrations, we recommend to extend the lag time between blister creation and the in vivo experiment from 1 hour to 12 to 24 hours in accordance with previous publications.[25,26]

Several difficulties were encountered during the in vivo application of sulfadiazine as an internal standard. Relative loss of sulfadiazine is not suitable for the calculation of the mucosal concentration of sulfadimidine. In fact, further research is necessary to investigate the suitability of sulfadiazine as an internal standard, in particular taking into account different experimental conditions from those in the present study (eg, higher concentrations in the perfusion solution). Otherwise, a better internal standard than sulfadiazine may need to be investigated.

Results of the present study indicated that in vivo microdialysis is suitable for continuous monitoring of unbound drug concentrations within the PSM of unsedated horses for 48 hours. Furthermore, this study has provided data regarding concentrations of sulfadimidine in the PSM of horses following IV administration of the drug. The study results could be applied to reevaluate existing treatment regimens involving sulfadimidine. Moreover, the method of intramucosal microdialysis could be applied to investigate local drug concentrations of other anti-infective agents used in the treatment of equine sinusitis.

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Footnotes

a. CMA 70 Brain Microdialysis Catheter, CMA Microdialysis AB, Solna, Sweden.
b. CMA 106 Syringe, CMA Microdialysis AB, Solna, Sweden.
c. CMA 107 Microdialysis Pump, CMA Microdialysis AB, Solna, Sweden.
d. Bigram, CP-Pharma Handelsgesellschaft mbH, Burgdorf, Germany.
e. 126 Solvent Module Pump, 507 Autosampler, 168 Detector, Beckmann, Munich, Germany.
f. 24 Karat, version 5.0, Merck, Darmstadt, Germany.
g. LichroCART 25-4 column (Rp18), Spark Holland, Emmen, The Netherlands.
h. LichroCART 4-A guard column, Spark Holland, Emmen, The Netherlands.
i. HPLC Column Heater, Spark Holland, Emmen, The Netherlands.
j. WinNonlin Professional Edition, version 5.3, Pharsight Corp, Mountain View, Calif.
k. Jllig H. *Beitrag zur Kenntnis der Nebenhöhlen der Nase der Haussäuger. Über den histologischen Aufbau der Nebenhöhlen der Nase bei den Haussäugetieren. Die Entwicklung der Nebenhöhlen-Systeme beim Rind*. PhD dissertation, Vereinigte Medizinische Fakultät der Grossherzoglich Hessischen Ludwigs-Universität zu Gießen, Gießen, Germany, 1910.

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