Pharmacokinetics and Body Fluid and Endometrial Concentrations of Ormetoprim-Sulfadimethoxine in Mares

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ABSTRACT

Six healthy adult mares were each given an oral loading dose of ormetoprim(OMP)-sulfadimethoxine (SDM) at a dosage of 9.2 mg of OMP/kg and 45.8 mg of SDM/kg, followed by four maintenance doses of 4.6 mg of OMP/kg and 22.9 mg of SDM/kg, at 24 h intervals. Ormetoprim and SDM concentrations were measured in serum, synovial fluid, peritoneal fluid, cerebrospinal fluid, urine and endometrium. The highest mean serum OMP concentration was 0.92 μg/mL 0.5 h after the first dose; the highest mean SDM concentration was 80.9 μg/mL 8 h after the first dose. The highest mean synovial fluid concentrations were 0.14 μg of OMP/mL and 28.5 μg of SDM/mL 12 h after the first dose. The highest mean peritoneal fluid concentrations were 0.19 μg of OMP/mL 6 h after the first dose and 25.5 μg of SDM/mL 8 h after the fifth dose. The highest mean endometrial concentrations were 0.56 μg of OMP/g and 28.5 μg of SDM/g 4 h after the fifth dose. The mean cerebrospinal fluid concentrations were 0.08 μg of OMP/mL and 2.1 μg of SDM/mL 5 h after the fifth dose. Mean trough urine drug concentrations were $\geq$ 0.4 μg of OMP/mL and $\geq$ 172 μg of SDM/mL. Two of the mares were each given a single intravenous (IV) injection of OMP and SDM at a dosage of 9.2 mg of OMP/kg and 45.8 mg of SDM/kg. Excitation and muscle fasciculations were observed in both mares after IV administration and all scheduled blood samples could be collected from only one of the two mares. Due to this drug reaction, IV experiments were not conducted in the four remaining mares. Serum concentrations of each drug were measured serially over a 24 h period in the one mare. For OMP, the mean overall elimination rate constant (K) was 0.40/h and the elimination half-life ($t_{1/2}$) was 1.7 h. The apparent volume of distribution (at steady state) was 1.19 L/kg and OMP clearance was 671 mL/h/kg. For SDM, K was 0.09/h and $t_{1/2}$ was 7.9 h. The apparent volume of distribution at steady state was 0.27 L/kg and SDM clearance was 25.0 mL/h/kg. A loading dose of 9.2 mg OMP/kg and 45.8 mg of SDM/kg, followed by a maintenance dose of 4.6 mg of OMP/kg and 22.9 mg of SDM/kg administered orally at 24 h intervals should be an appropriate oral dosage regimen for OMP/SDM paste for the treatment of bacterial infections in horses.

RÉSUMÉ

Cette expérience portait sur six juments adultes qui reçurent une dose buccale d'ormétoprime et de sulfadiméthoxine, dans les proportions respectives de 9,2 et 45,8 mg/kg. On leur administra ensuite, à 24 heures d'intervalle, quatre doses d'entretien, dans les proportions respectives de 4,6 et 22,9 mg/kg. On détermina ensuite la concentration d'ormétoprime et celle de sulfadiméthoxine dans le sérum, la synovie, les liquides péritonéal et céphalo-rachidien, l'urine et l'endomètre. La concentration sérique moyenne la plus élevée d'ormétoprime atteignit 0,92 μg/mL, 30 minutes après la première dose, alors que celle de la sulfadiméthoxine atteignit 80,9 μg/mL, huit heures après la première dose. Les concentrations moyennes les plus élevées de la synovie en ormétoprime et en sulfadiméthoxine atteignirent respectivement 0,14 et 28,5 μg/mL, 12 heures après la première dose. La concentration moyenne la plus élevée du liquide péritonéal en ormétoprime atteignit 0,19 μg/mL, six heures après la première dose, tandis que celle de la sulfadiméthoxine atteignit 25,5 μg/mL, huit heures après la cinquième dose. Les concentrations moyennes les plus élevées de l'endomètre en ormétoprime et en sulfadiméthoxine atteignirent respectivement 0,56 et 28.5 μg/mL, quatre heures après la cinquième dose. Les concentrations moyennes du liquide céphalo-rachidien en ormétoprime et en sulfadiméthoxine atteignirent respectivement 0,08 et 2,1 μg/mL, cinq heures après la cinquième dose. Les concentrations moyennes d'ormétoprime et de sulfadiméthoxine éliminées dans l'urine atteignirent respectivement $\geq$ 0,4 et $\geq$ 172 μg/mL.

Deux des juments reçurent une seule injection intraveineuse d'ormétoprime et de sulfadiméthoxine, aux doses respectives de 9,2 et 45,8 mg/kg. Elles manifestèrent de l'excitation et des fasciculations musculaires qui ne rendirent possible le prélèvement de tous les échantillons de sang prévus,

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que chez l'une d'elles. À cause de cette réaction, on annula les injections intraveineuses prévues pour les quatre autres juments. On mesura ensuite la concentration de chacune des deux drogues précitées, à divers intervalles d'une période de 24 heures, chez une jument. La constante du taux moyen de l'élimination totale de l'ormétoprime fut de 0,4 h et sa demi-vie, 1,7 h, alors que son volume apparent de distribution, à l'état stable, s'établit à 1,19 L/kg et sa clairance, à 671 mL/h/kg. Quant à la sulfadiméthoxine, la constante de son taux moyen d'élimination totale fut de 0,09 h et sa demi-vie, de 7,9 h. Son volume apparent de distribution, à l'état stable, s'établit à 0,27 L/kg et sa clairance, à 25 mL/h/kg. Une dose buccale initiale de pâte d'ormétoprime et de sulfadiméthoxine, dans les proportions respectives de 9,2 et 45,8 mg/kg, suivie d'une dose d'entretien, dans les proportions respectives de 4,6 et 22,9 mg/kg, à 24 heures d'intervalle, devrait correspondre à une quantité suffisante pour le traitement des infections bactériennes, chez le cheval.

INTRODUCTION

Ormetoprim(OMP)-sulfadimethoxine(SDM) preparations have been used in the treatment of bacterial infections in poultry (1,2), swine (3), and cattle (4). The pharmacokinetic properties of this drug combination after single intravenous (IV) and oral administration to calves have been described; SDM was well absorbed from the gastrointestinal tract, but OMP was not (5). Serum concentrations and pharmacokinetics of SDM after IV or oral administration to adult horses have been reported (6,7); the elimination half-life ($t_{½}$) and apparent volume of distribution values were 11.3 h and 1.95 L/kg, respectively (7). Sulfadimethoxine distributes readily into all body fluids and tissues after IV administration to horses (8). After oral administration of OMP/SDM paste to foals seven to nine weeks of age, $t_{½}$ values for OMP and SDM were 1.45 h and 13.53 h, respectively (9).

Purposes of the present study were to determine the distribution of OMP/SDM in body fluids and endometrial tissue after repeated oral administration, and to determine the pharmacokinetics of OMP/SDM in mares given the drug combination IV.

MATERIALS AND METHODS

MARES

Six healthy adult mares, weighing from 489 to 527 kg, were used. A physical examination and complete blood count were done on each mare before each experiment. They received no medication for a minimum of two months prior to experiment 1. They were housed in facilities accredited by the American Association of Laboratory Animal Care, and all procedures were approved by the University of Florida Animal Care and Use Committee, in compliance with National Institutes of Health guidelines.

EXPERIMENTAL DESIGN

Experiment 1 — To each of the six mares, OMP/SDM paste (Roche Laboratories, Nutley, New Jersey; 90 mg of OMP/g and 450 mg of SDM/g) was administered orally in a loading dose of 9.2 mg of OMP/kg and 45.8 mg of SDM/kg, followed by four maintenance doses (4.6 mg of OMP/kg and 22.9 mg of SDM/kg) at 24 h intervals. Blood samples were collected by needle venipuncture from the jugular veins. Synovial fluid samples were collected by needle arthrocentesis. Peritoneal fluid samples were obtained by inserting a teat cannula through a stab incision in the linea alba. Cerebrospinal fluid (CSF) was collected by placing a 17.7 cm x 18 gauge spinal needle in the lumbosacral space. A uterine biopsy forcep was used to obtain endometrial tissue and urine specimens were collected with a metal mare catheter (10,11), according to the schedule in Tables I and II.

Experiment 2 — Approximately five weeks after experiment 1, OMP (Roche Laboratories; 50 mg/mL in 0.3 *M* acetic acid) and SDM (Albon, Roche Laboratories; 400 mg of SDM/mL) were administered IV, sequentially, in a single dose of 9.2 mg of OMP/kg and 45.8 mg of SDM/kg through a 13.3 cm x 16 gauge Teflon catheter (Angiocath, Deseret Co., Sandy, Utah) to two of the mares. From only one of the mares, blood samples were collected from the other jugular vein at 0, 2, 4, 7, 10, 15, 20, 30 and 45 min and at 1, 1.5, 2, 3, 4, 6, 8, 10, 12 and 24 h after injection.

DRUG ASSAYS

Fluid samples were centrifuged at 510 x *g* for 10 min, and the supernate decanted and frozen at -20°C until assayed. The endometrial biopsies were also frozen. At the time of assay, the endometrial tissue was weighed and homogenized with saline in a tissue grinder (Fisher Scientific Co., Pittsburg, Pennsylvania). This suspension was centrifuged at 510 x *g* and the supernate collected for assay.

Ormetoprim and SDM concentrations were determined by high performance liquid chromatography (HPLC), by modification of a previously described method (12). Serum, synovial, peritoneal, CSF, endometrial and urine specimens were prepared by adding 200 μL of 0.5 *M* tetrabutylammonium hydroxide and 1 mL of 0.05 *M* buffer solution (pH 10; Fisher Scientific Co.) and then vortexed for 10 s. After addition of 4 mL of methylene chloride, the mixture was agitated on an Eberbach horizontal shaker (Fisher Scientific Co.) for 2 min. Each sample was then centrifuged at 3322 x *g* for 15 min at 10°C. The aqueous layer was discarded and 500 μL of the organic phase were injected into the HPLC.

The HPLC consisted of a single piston reciprocating pump, a variable wavelength detector operated at 288 nm (Rainin Instrument Co., Woburn, Massachusetts), and a silica column (Fisher Scientific Co.). The mobile phase was a mixture of 976 mL of chloroform, 50 mL of methanol, 2 mL of H_2O, and 0.6 mL of concentrated NH_4OH, with a flow rate of 1.5 mL/min. Under these conditions, retention times for OMP and SDM were about 6 and 9 min, respectively.

Ormetoprim-sulfadimethoxine standards were prepared by adding OMP/SDM to horse serum or urine; standards were extracted in the same way as the unknown samples. Standard curves were prepared by linear regression analysis using the area under the absorbance-vs-time curve compared with OMP and SDM concentrations. Ormetoprim and SDM concentrations in the unknown samples were determined from the standard curve. The lowest limit of the assay was 0.02 μg of OMP/mL and 0.05 μg of SDM/mL.

DATA ANALYSIS

For experiment 1 (oral), overall mean serum concentrations of OMP

TABLE I. Mean OMP Concentrations in Six Mares Given a Loading Dose of OMP/SDM Paste (92.5 mg of OMP/kg and 45.8 mg of SDM/kg), Followed by Four Maintenance Doses (4.6 mg of OMP/kg and 22.9 mg of SDM/kg) Orally at 24 h Intervals (Experiment 1)

Time (h)	Mean (±SD) OMP Concentrations (μg/mL)					
	Serum	Synovial Fluid	Peritoneal Fluid	CSF	Endometrium	Urine
0[a]	0	...	0	...	0	0
0.25	0.29 ±0.298	...	...	...	...	...
0.5	0.92 ±1.045	...	...	...	...	...
0.75	0.89 ±0.872	...	...	...	...	...
1	0.81 ±0.704	...	...	...	...	...
1.5	0.56 ±0.461	...	...	...	...	...
2	0.43 ±0.409	...	...	...	...	...
3	0.26 ±0.219	...	...	...	...	...
4	0.21 ±0.170	0.14±0.122	0.13±0.116	...	...	...
5	0.17 ±0.152	...	...	...	...	...
6	0.16 ±0.112	0.10±0.084	0.19±0.254	...	...	5.7±3.94
8	0.08 ±0.074	0.12±0.143	0.12±0.079	...	...	...
12	0.09 ±0.150	0.02±0.020	0.01±0.022	...	...	3.1±3.31
24[a]	0.005±0.012	NM	0.02±0.032	...	...	0.7±0.73
30	0.08 ±0.028	...	...	...	...	...
48[a]	0.003±0.007	...	...	...	...	...
54	0.14 ±0.074	...	...	...	...	...
72[a]	0.01 ±0.023	...	...	...	...	...
78	0.12 ±0.086	...	...	...	...	...
96[a]	0.006±0.015	...	0.01±0.028	...	...	0.9±0.72
96.25	0.05 ±0.060	...	...	...	...	...
96.5	0.39 ±0.397	...	...	...	...	...
96.75	0.59 ±0.564	...	...	...	...	...
97	0.64 ±0.517	...	...	...	...	...
97.5	0.44 ±0.297	...	...	...	...	...
98	0.31 ±0.217	...	...	...	...	...
99	0.17 ±0.124	...	...	...	...	...
100	0.13 ±0.094	0.09±0.075	0.08±0.046[b]	...	0.56±0.487	...
101	0.17 ±0.162	...	...	0.08±0.056[b]	...	...
102	0.14 ±0.095	0.10±0.087	0.07±0.061	...	...	4.4±3.27
104	0.07 ±0.025	0.04±0.047	0.04±0.058	...	0.11±0.139	...
108	0.08 ±0.094	0.02±0.024	0.02±0.046[b]	...	0.04±0.109	2.4±1.79
120	0.03 ±0.052	NM	NM	...	...	0.4±0.18

[a]Specimens collected immediately before administration of OMP/SDM
[b]n = 5
NM = concentrations below measurable values
. . . = no sample

and SDM were determined for the 24 h after the first and fifth doses in each of the six mares. These values were calculated as the area under the serum concentration-vs-time curve (AUC) for the drug during that period (0 to 24 h, and 96 to 120 h) divided by the time (24 h). The AUC was determined by the trapezoidal rule (13). Significant differences between overall mean OMP and SDM serum concentrations after the first and fifth doses were determined by using the paired Student's *t*-test ($p < 0.05$).

For experiment 2 (IV), serum OMP concentration-vs-time and serum SDM concentration-vs-time were estimated as a three compartment model using the equation:

$$C_s = C_1 \cdot e^{-\lambda_1 \cdot t} + C_2 \cdot e^{-\lambda_2 \cdot t} + C_3 \cdot e^{-\lambda_3 \cdot t} \quad (1)$$

where C_s is the serum OMP or SDM concentration; C_1, C_2 and C_3 are the intercepts for the three components; λ_1, λ_2 and λ_3 are the slopes of the components; and t is time in h. The equation was fitted to the serum concentration-vs-time data, using a digital computer program that minimized the sum of the squared deviations (14).

The overall elimination rate constant (K) was equated to λ_3. The elimination half-life ($t_{1/2}$) was calculated as the ratio of the natural logarithm of 2 to K. AUC after IV injection was calculated from:

$$AUC = C_1/\lambda_1 + C_2/\lambda_2 + C_3/\lambda_3 \quad (2)$$

The apparent volume of distribution based on area under serum concentration-vs-time curve, ($V_{d(area)}$), and volume of distribution at steady state ($V_{d(ss)}$) were estimated using equations 3 and 4, respectively:

$$V_{d(area)} = dose/AUC/K \quad (3)$$

$$V_{d(ss)} = dose \cdot AUMC/AUC^2 \quad (4)$$

where AUMC is the first moment of the serum concentration-vs-time curve, and was calculated after IV administration from:

$$AUMC = C_1/\lambda_1^2 + C_2/\lambda_2^2 + C_3/\lambda_3^2 \quad (5)$$

Total serum clearance was calculated as dose divided by AUC.

RESULTS

Before each experiment, all physical and clinical laboratory findings for the mares were normal.

Body fluid and endometrial concentrations of OMP and SDM determined from experiment 1 (oral) are shown in Tables I and II. After oral administration, mean serum OMP concentrations peaked at 0.5 to 0.75 h and mean serum SDM concentrations peaked at 5 to 8 h. Overall mean OMP serum concentrations after the first and fifth doses were not significantly different ($p > 0.05$), with respective values of 0.11 ± 0.038 μg/mL and 0.11 ± 0.026 μg/mL. Similarly, the overall mean SDM serum concentrations after the first and fifth doses were not significantly different ($p > 0.05$), with respective values of 68 ± 7.1 μg/mL and 72 ± 6.8 μg/mL.

The highest measured mean synovial OMP and SDM concentrations were at approximately 4 h and at 6 to 12 h, respectively, after oral administration. The highest measured mean peritoneal OMP and SDM concentrations were at approximately 4 to 6 h and at 8 to 12 h, respectively, after oral administration (Tables I and II).

The highest mean endometrial OMP and SDM concentrations were 0.56 μg/g and 28.5 μg/g, respectively, 4 h after the fifth dose (Tables I and II). In the five mares in which CSF samples were obtained, the mean OMP and SDM concentrations in CSF were 0.08 μg/mL and 2.1 μg/mL, respectively, 5 h after the fifth dose.

The mean urine OMP concentration was 5.7 μg/mL at 6 h after the first

dose; mean trough concentrations in urine were ≥0.4 μg/mL. The highest mean urine SDM concentration was 544 μg/mL 12 h after the first dose; mean trough concentrations in urine were ≥172 μg/mL (Tables I and II).

In experiment 2 (IV), at approximately 6 min after injection, one of the two mares became excited and showed signs of anxiety, generalized muscle fasciculations and profuse sweating, and therefore blood samples were not collected from the mare until the 1.5 h sampling time. The other mare showed similar, but milder signs and could be safely approached for all samples. Both mares appeared normal within 5 to 6 h. Because of the reaction observed in these two mares, no further IV injections were attempted. The serum OMP concentration in the mare in which all samples could be collected was 38.9 μg/mL 2 min after injection, and declined to 0.04 μg/mL 12 h after injection. The serum SDM concentration was 67.07 μg/mL 2 min after injection and declined to 0.05 μg/mL at 24 h. The $t_{1/2}$ values for OMP and SDM were 1.71 h and 7.90 h, respectively (Table III).

TABLE II. Mean SDM Concentrations in Six Mares Given a Loading Dose of OMP/SDM Paste (92.5 mg of OMP/kg and 45.8 mg of SDM/kg), Followed by Four Maintenance Doses (4.6 mg of OMP/kg and 22.9 mg of SDM/kg) Orally at 24 h Intervals (Experiment 1)

Time (h)	Mean (±SD) SDM Concentrations (μg/mL)					
	Serum	Synovial Fluid	Peritoneal Fluid	CSF	Endometrium	Urine
0[a]	0	...	0	...	0	0
0.25	3.6± 3.25	...	...	...	...	...
0.5	17.4±10.48	...	...	...	...	...
0.75	33.9±18.05	...	...	...	...	...
1	45.5±21.57	...	...	...	...	...
1.5	61.4±25.69	...	...	...	...	...
2	72.9±28.18	...	...	...	...	...
3	76.0±18.57	...	...	...	...	...
4	75.5±25.08	19.0±6.44	20.3± 4.29	...	...	...
5	76.9±18.06	...	...	...	...	...
6	79.2±17.53	20.8±6.44	24.7± 3.95	...	...	499±220.7
8	80.9±19.19	22.4±9.95	25.3± 5.45	...	...	...
12	76.3±17.60	28.5±9.84	25.4± 5.62	...	...	544±316.7
24[a]	54.5±19.51	18.1±4.47	17.5± 5.60	...	...	347±333.7
30	84.2±13.77	...	...	...	...	...
48[a]	41.6± 9.99	...	...	...	...	...
54	84.4±13.40	...	...	...	...	...
72[a]	39.8±12.74	...	...	...	...	...
78	81.2±14.81	...	...	...	...	...
96[a]	43.7±11.02	...	14.1± 3.54	...	...	172±135.0
96.25	46.4±12.78	...	...	...	...	...
96.5	54.6±17.49	...	...	...	...	...
96.75	60.3±18.38	...	...	...	...	...
97	68.0±22.67	...	...	...	...	...
97.5	76.2±23.67	...	...	...	...	...
98	77.6±22.67	...	...	...	...	...
99	75.8±22.10	...	...	...	...	...
100	78.0±24.47	19.2±8.82	24.0± 9.98[b]	...	28.5±10.24	...
101	79.0±25.11	...	...	2.1±0.77[b]	...	...
102	78.6±27.17	20.6±8.32	24.1±12.83	...	...	363±230.4
104	76.8±27.89	20.4±8.23	25.5± 8.43	...	24.1±13.95	...
108	75.9±19.45	19.1±6.34	21.7± 6.75[b]	...	19.6±12.29	347±302.4
120	60.1±13.97	13.6±3.63	19.1± 6.51	...	...	300±210.7

[a]Specimens collected immediately before administration of OMP/SDM
[b]n = 5
NM = concentrations below measurable values
... = no sample

DISCUSSION

Absorption of both drugs was highly variable, as seen from high SD values in Tables I and II. Serum concentration-vs-time curves for each mare did not show a definite peak or decline phase. As a result, pharmacokinetic values for each mare could not be determined from experiment 1 and therefore only overall mean serum drug concentrations from the first and fifth doses were compared. The observation that the overall mean OMP and SDM serum concentrations were not significantly different ($p > 0.05$) after the first and fifth doses would indicate that the loading dose used was appropriate.

Synovial fluid OMP and SDM concentrations were similar to concurrent peritoneal fluid concentrations at all sampling times (Tables I and II). At 3 h after IV injection of SDM (40 mg/kg) to one horse, Oh-Ishi (8) observed that synovial and peritoneal fluid SDM concentrations (51.8 μg/mL and 58.3 μg/mL, respectively) were approximately 40% of the concurrent serum SDM concentration (133.0 μg/mL); the concurrent SDM concentration in CSF was 30.0 μg/mL. Although OMP penetration of CSF in our study was similar to that reported for trimethoprim in mares, SDM did not enter the CSF as well as sulfamethoxazole (15).

Neither OMP nor SDM concentrated in endometrial tissue as has been reported for trimethoprim and sulfamethoxazole in mares (15); mean OMP endometrial concentrations were greater than the concurrent serum concentrations at 4 and 8 h after the fifth dose (Table I), whereas mean SDM endometrial concentrations were lower than concurrent serum concentrations at all sampling times during the fifth dose interval (Table II). Because mean peak OMP serum concentrations occurred at 0.5 to 1 h after oral administration (Table I), it is possible that the earliest synovial, peritoneal, CSF and endometrial samples were collected too late to observe peak OMP concentrations in these fluids and tissue.

In experiment 2, the systemic reaction observed after IV injection of OMP/SDM was presumed to be a response to OMP because the SDM IV preparation used in this study is approved for use in horses. In a similar study in calves (5), in which a lower dosage was used (5.5 mg/kg), no adverse reaction was reported. The OMP clearance for the one mare in which all samples were collected was similar to that reported for trimethoprim in mares (886 mL/h/kg) (15). Reported $t_{1/2}$ values for trimethoprim

TABLE III. Pharmacokinetic Values of OMP and SDM in a Mare Given 9.2 mg of OMP/kg and 45.8 mg of SDM/kg, IV

Pharmacokinetic Value	OMP	SDM
K (/h)	0.40	0.09
$t_{1/2}$ (h)	1.72	7.90
$V_{d(area)}$ (L/kg)	1.66	0.28
$V_{d(ss)}$ (L/kg)	1.19	0.27
Clearance (mL/h/kg)	671	25.0

K = overall elimination rate constant; $t_{1/2}$ = elimination half-life; $V_{d(area)}$ = apparent volume of distribution based on area under serum concentration-vs-time curve; $V_{d(ss)}$ = apparent volume of distribution at steady state

after IV administration range from 1.9 to 3.2 h for horses (15-18), compared to 1.7 h for OMP in the present study. Reported values for $V_{d(area)}$ after IV administration of trimethoprim range from 0.59 to 2.3 L/kg (15-18), compared to 1.66 L/kg for OMP in our study. For IV administered SDM in horses, Durr (7) reported $t_{1/2}$ and $V_{d(area)}$ values of 11.3 h and 1.95 L/kg, respectively, compared with 7.90 h and 0.28 L/kg in our study. Because SDM was found in high concentrations in bile after IV administration to horses, it has been suggested that the drug may undergo enterohepatic circulation (8).

In a previous report (19), minimum inhibitory concentration (MIC) values for OMP/SDM for 90% of isolants of equine origin were: *Escherichia coli* = 2.0/38 μg/mL; *Corynebacterium (Rhodococcus) equi* = 1.0/19 μg/mL; *Streptococcus equi* = 0.5/9.5 μg/mL; *Corynebacterium pseudotuberculosis, Staphylococcus* sp, and *Streptococcus zooepidemicus* ≤ 0.25/4.75 μg/mL. Except for *E. coli*, these MIC values were identical to those reported for trimethoprim/sulfadiazine (19); similarly, trimethoprim/sulfadiazine and trimethoprim/sulfamethoxazole yield equivalent *in vitro* bacterial sensitivity results (20,21). Ormetoprim/sulfonamide and trimethoprim/sulfonamide combinations used for *in vitro* tests are in the optimum ratio (1:20), which allows the maximum effect on the organism by the lowest concentration of each drug. However, synergism occurs over a wide range of ratios in which a lower concentration of one of the components may be offset by a higher concentration of the other component (22). Ormetoprim/sulfadimethoxine ratios in the body fluids and endometrium of our mares were much less than 1:20 at almost all sampling times. Because OMP appears to be absorbed less readily than SDM in horses, perhaps the OMP/SDM paste should have a higher OMP/SDM ratio. From a practical and therapeutic standpoint, however, this may not make a difference in the clinical efficacy of the drug (22).

Based on MIC values for equine pathogens (19), the oral dosage of OMP/SDM used in this study should be adequate for the treatment of infections caused by *C. pseudotuberculosis, S. aureus* and *S. zooepidemicus*. Although higher dosages may be necessary to treat infections caused by other organisms, further clinical trials may be necessary to determine the optimal oral dosage of OMP/SDM paste needed for horses. Based on our present findings, however, we think that a loading dose of 9.2 mg OMP/kg and 45.8 mg of SDM/kg, followed by a maintenance dose of 4.6 mg of OMP/kg and 22.9 mg of SDM/kg administered orally at 24 h intervals should be an appropriate oral dosage regimen for OMP/SDM paste for the treatment of bacterial infections in horses.

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