Pharmacokinetics of sulfadimethoxine and ormetoprim in a 5:1 ratio following intraperitoneal and oral administration, in the hybrid striped bass (*Morone chrysops* × *Morone saxitalis*)

R. S. BAKAL*
S. A. BAI† &
M. K. STOSKOPF*

**Environmental Medicine Consortium and Department of Clinical Sciences, North Carolina State University, College of Veterinary Medicine, Raleigh, NC; †Dupont Pharmaceuticals Co., Newark, DE, USA*

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Selected pharmacokinetic parameters for sulfadimethoxine and ormetoprim, administered in a 5:1 ratio, via the oral and intraperitoneal (i.p.) routes were determined in the hybrid striped bass (*Morone chrysops* × *Morone saxitalis*). Plasma concentrations of both drugs were determined by high-performance liquid chromatography. A first-order one-compartment model adequately described plasma drug disposition. The elimination half-lives for sulfadimethoxine following i.p. and oral administration were 26 and 10.5 h, respectively. The half-lives for ormetoprim administered via i.p. and oral routes were 7.5 and 3.9 h, respectively. C_{max} for sulfadimethoxine via the i.p. and oral routes were calculated to be 27.7 (±9.0) μg/mL at 3.6 h and 3.2 (±1.2) μg/mL at 1.2 h, respectively. C_{max} for ormetoprim via the i.p. route was calculated to be 1.2 (±0.5) μg/mL at 9.1 h and 1.58 (±0.7) μg/mL at 5.7 h for the oral route. The oral availability of sulfadimethoxine relative to the i.p. route was 4.6%, while the oral availability of ormetoprim relative to the i.p. route was 78.5%. Due to the nonconstant ratio of these drugs in the plasma of the animal, the actual drug ratio to use for determining minimum inhibitory concentration (*MIC*) is unclear. Using the ratio of the total amount of each drug that is absorbed as a surrogate for the mean actual ratio may be the best alternative to current methods. Using this ratio as determined in these studies, (2.14:1 sulfadimethoxine:ormetoprim) to determine the *MIC*s the single 50 mg/kg oral dose of the 5:1 combination of sulfadimethoxine and ormetoprim appears to provide plasma concentrations high enough to inhibit the growth of *Yersinia ruckeri*, *Edwardsiella tarda*, and *Escherichia coli*.

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Robert S. Bakal, US Fish and Wildlife Service, Warm Springs Regional Fish Health Center, 5308 Spring St., Warm Springs, GA 31830, USA. E-mail: robert_bakal@fws.gov

INTRODUCTION

Sulfadimethoxine and ormetoprim are each individually effective against many pathogenic organisms. However, given together, a synergistic effect makes the combination more effective than either compound alone (Beam, 1992). A 5:1 sulfadimethoxine:ormetoprim combination is approved, by the US Food and Drug Administration, for treatment of *Edwardsiella ictaluri* infections in channel catfish (*Ictalurus punctatus*) and *Aeromonas salmonicida* infections in rainbow trout (*Oncorhynchus mykiss*). The drug combination is also used in an extra label manner to treat many other diseases in a variety of fish species including the hybrid striped bass (*Morone chrysops* × *Morone saxitalis*), an economically important food fish. While the combination of sulfadimethoxine and ormetoprim has been used in this species for quite some time, there are currently no pharmacokinetic data available to determine if dosing protocols being used are effective.

Kinetic parameters for sulfadimethoxine and ormetoprim given individually have been determined in rainbow trout (Kleinow *et al.*, 1992; Kleinow & Lech, 1988; Droy *et al.*, 1990), channel catfish (Squibb *et al.*, 1988; Michel *et al.*, 1990; Plakas *et al.*, 1990), and Chinook salmon (*Oncorhynchus tshawytscha*) (Walisser *et al.*, 1990). Studies of the pharmacokinetic parameters for the two drugs in combination are much

rare, being limited to intravenous and oral dosing experiments in rainbow trout (Droy *et al.*, 1989). It is possible the lack of studies of this nature could be due to problems in achieving a combined drug solution suitable for intravenous administration. Because of the chemical nature of the two drugs, a suspension, rather than a solution, must be used. In our laboratory, injection of suspensions into the vasculature via the sinus venosus of the hybrid striped bass (Bakal *et al.*, 1999) resulted in acute damage to the gills, hemorrhage, and death by exsanguination. For this reason, we chose intraperitoneal (i.p.)administration of the drug in an effort to approximate total bioavailability and allow for the calculation of a relative oral availability.

The purpose of this study was to determine kinetic parameters of a 5:1 combination of sulfadimethoxine and ormetoprim after i.p. and oral dosing in the hybrid striped bass. This data can be used in conjunction with bacterial minimum inhibitory concentration (*MIC*) data to predict effective treatment protocols for infected hybrid striped bass.

MATERIALS AND METHODS

Animals

Hybrid striped bass weighing between 565 and 805 g were obtained from a commercial grow-out facility (Carolina Fisheries, Aurora, NC, USA). The fish were maintained in a 1200-L fiberglass tank for a period of 30 days prior to the study. During the study each fish was moved to an individual 120-L fiberglass tank. All tanks were maintained as flow through systems with supplemental aeration, and shared a common water supply. Water temperature remained between 16 and 17 °C for the duration of the study. Fish were fed approximately 1% of their body weight per day of #3 commercial trout pellets (Zeigler Brothers, Gardners, PA, USA).

Surgery

Twenty-one hybrid striped bass were randomly divided into two groups, 12 in group A and nine in group B. Each fish was anesthetized in 10 L of a 200 mg/L solution of tricaine methanesulphonate (MS-222) (Argent, Redmond, WA, USA) and transferred to a recirculating anesthesia machine (Bakal & Stoskopf, 1994) containing 10 L of an 80 p.p.m. solution of MS-222. Catheters were placed in the sinus venosus of each fish and a 46 cm extension set, terminated with a 3-way stopcock, was attached. A 3-mL syringe containing heparinized saline used to flush the catheter was attached to one port of the stopcock, and a second 3-mL syringe filled with air was attached to the second port to serve as a float (Bakal *et al.*, 1999).

Fish in the i.p. portion of the study (group A) were also fitted with i.p. catheters placed in the anesthetized fish by inserting a 20-gauge × 2.5 cm over the needle Teflon® catheter (Johnson & Johnson Medical, Arlington, TX, USA) in the ventral third of the lateral aspect of the fish immediately cranial to the cloaca. Catheter placement was checked by applying gentle negative pressure with a 3-mL syringe and the catheter is sutured in place using 2-0 nylon in a simple interrupted pattern. A 46 cm extension set was attached to the catheter, the joint covered with epoxy resin to prevent slipping, and the extension set sutured to the skin of fish using 2-0 nylon in a Chinese finger trap suture pattern. A 3-mL syringe on the terminal end of the extension set was kept afloat by attaching it, with a rubber band, to the air-filled syringe on the end of the vascular catheter's extension set. Ten percent povidone iodine ointment was swabbed onto the sites where the catheter and sutures penetrate the skin to help prevent infection.

The fish in the oral dosing study (group B) were fitted with indwelling stomach tubes while under anesthesia for vascular catheterization. Indwelling stomach tubes were made by fitting 23-gauge luer adapter (Becton Dickinson, Rutherford, NJ, USA) into one end of a 61 cm length of Teflon tubing (1.6 mm i.d., 3.2 mm o.d.) (Fisher Scientific, Norcross, GA, USA). The joint between the tubing and the hub was glued with two-part epoxy (Duro; Lock-tite Corp, Rocky Hill, CT, USA) to prevent accidental removal of the hub. A small incision was made in the thin membrane immediately caudal to the left commissure of the mouth, of the anesthetized fish, with a #10 scalpel blade. The open end of the tube was passed through this incision into the mouth. The tube was then passed through the esophagus into the stomach of fish and placement checked by flushing 2 mL of water through the tube and monitoring for return around the tube. A tape tag, applied approximately 10–15 cm from the distal end of the tube, was sutured to the roof of the mouth using two simple interrupted 2-0 nylon sutures. The distal end of the tube was terminated with a 3-mL syringe, which was attached with a rubber band to the air filled syringe at the end of the extension set on the vascular catheter. Ten percent povidone iodine ointment was swabbed onto the incision and the suture sites.

All fish were allowed to recover from the anesthetic procedures for 7 days prior to the initiation of the kinetic studies.

Experimental design

A 5:1 solution of the two drugs for delivery to the fish was made by adding 42 mg/mL sulfadimethoxine and 8 mg/mL ormetoprim to a 40% propylene glycol, 10% ethanol solution. The 12 fish in group A each received 50 mg/kg (1 mL/kg) via the i.p. catheter. The nine fish in group B each received the same dose via the indwelling stomach tube. Stomach tubes and i.p. catheters were flushed with 1-mL sterile saline immediately after drug administration. Prior to sample acquisition, 1 mL of blood was drawn through the catheter and extension set to prevent dilution of the samples with heparinized saline. This blood was returned to the fish immediately after sample collection. Blood samples (0.75 mL) were collected from each group A fish at the three of the following time points: 0, 0.25, 2, 4, 6, 8, 12, 18, 24, 36, 48, and 96 h postdrug administration. Similarly, each group B fish was sampled at three of the following points: 0, 0.25, 2, 4, 6, 8, 12, 18, and 48 h postdrug administration. This sampling regimen resulted in a total of three samples being taken from three separate fish at each time

point and a total of three samples collected from each individual fish. The total blood volume removed from each fish was 2.25 mL.

Sample preparation

Blood samples were centrifuged within 10 min of collection. Plasma was stored at −70 °C until sample analysis could be performed. To extract the drugs from plasma, 250 μL of 5 N sodium hydroxide was added to 250 μL of plasma and shaken vigorously for 2 min. A pair of 2 mL chloroform extractions, of the resulting mixture, each agitated for 30 min and centrifuged at 1000 ***g*** for 10 min were performed. The chloroform fractions containing the ormetoprim were combined, dried under nitrogen, and rehydrated with 1.25 mL of mobile phase. Duplicate 2 mL acetone extractions of the remaining aqueous fraction, containing the sulfadimethoxine, were vortexed for 2 min, centrifuged for 30 min at 5000 ***g*** and combined, dried under nitrogen, and rehydrated in 1.25 mL of mobile phase. Twenty microliters of each rehydrated fraction was injected in the high-performance liquid chromatography (HPLC). Individual analysis of the two fractions was necessitated by the presence of interfering substances, which had similar retention times to ormetoprim, in the sulfadimethoxine fraction.

Sample analysis

All samples were analyzed by HPLC using a previously developed method (Bakal & Stoskopf, 2001). The HPLC system consisted of a Waters 600E multisolvent delivery system (Waters Corp., Milford, MA, USA) equipped with a Shimadzu SIL-9a auto injector (Shimadzu Corporation, Kyoto, Japan). The stationary phase consisted of a Supelcosil LC-18DB column (25 cm × 2.1 mm, pore size = 5 μm; Supelco, Bellafonte, PA, USA), preceded by a column inlet filter (0.5 μm × 3 mm; Rheodyne, Cotati, CA, USA), and a Supelguard LC-18DB guard column (2 cm × 2.1 mm; Supelco). The mobile phase consisted of an 80:15:5 mixture of running buffer, acetonitrile, and methanol. The running buffer was: 0.1 M potassium phosphate, 50 mM triethanolamine, and 1% acetic acid, adjusted to pH 3 with phosphoric acid. Elution was isocratic at a flow rate was 0.5 mL/min at room temperature. UV absorption was monitored at 288 nm using a LDC Analytical SpectroMonitor 3100 (LDC Analytical, Riviera Beach, FL, USA).

Chromatographic data acquisition, storage, and analysis were achieved with SMAD Data System (Morgan-Kennedy Research, College Station, TX, USA) and a Macintosh IIci computer (Apple, Cupertino, CA, USA).

The mean recoveries of sulfadimethoxine and ormetoprim were 92 and 96%, respectively. Total run time was 25 min per injection. Ormetoprim eluted from the column at 4.2 min, while sulfadimethoxine eluted at 18.5 min. Sulfadimethoxine and ormetoprim were used as external standards. Five-point standard calibration curves were created for ormetoprim over a range of 0–10 μg/mL and sulfadimethoxine over a range of 0–50 μg/mL. Regression analysis of the two curves was performed (Excel 5.0; Microsoft Corporation, Redmond, WA, USA) and the equations of the regression lines obtained (sulfadimethoxine: $y = 187607.3x + 6706.1$, ormetoprim: $y = 82192.4x + 363.7$), with a correlation coefficient $R^2 = 0.98$. The regression line equations, where y = area under the curve, were used to calculate drug concentrations in the experimental samples. The limit of detection, defined as three times the height of baseline noise, for both drugs was 25 ng/mL.

Pharmacokinetic calculations

Pkanalyst software (MicroMath Scientific Software, Salt Lake City, UT, USA) was used to generate pharmacokinetic data for both the i.p. and oral routes of administration. The data was fitted to a single-compartment model, characterized by the equation:

$$C_t = \frac{\text{Dose} \times K_{ab}}{V_d \times (K_{ab} - K_{elim})} \times \left(e^{-K_{elim} \times t} - e^{-K_{ab} \times t}\right),$$

where C_t is the plasma concentration at time t, V_d is the volume of distribution, K_{ab} is the rate of absorption, and K_{elim} is the rate of elimination. Calculations of the elimination and absorption half-lives and area under the time–concentration curve (*AUC*) were made using the mean concentration at each time point. The relative availability, C_{max}, and t_{max} of the drugs were calculated from the following equations:

$$F_{relative} = \frac{AUC_{oral}}{AUC_{i.p.}} \times \frac{\text{Dose}_{i.p.}}{\text{Dose}_{oral}},$$

$$C_{max} = \frac{\text{Dose}}{V_d} \times (K_{elim}/K_{ab})^{K_{elim}/(K_{ab} - K_{elim})},$$

$$t_{max} = \frac{1}{K_{ab} - K_{elim}} \times \ln \frac{K_{ab}}{K_{elim}}.$$

MIC determination

The *MIC*s of sulfadimethoxine and ormetoprim, as well as 5:1 and 2.14:1 combinations of these two drugs, were determined for six bacterial isolates. The 2.14:1 ratio is the ratio of the calculated areas under the curve for each drug. This ratio provides an average ratio of the drugs over time and may more accurately depict the *in vivo* situation as it takes into account the kinetics of the drug. The organisms for which the *MIC*s were determined were *Aeromonas salmonicida*, isolated from a *Chinook salmon*, *Aeromonas hydrophila*, and *Edwardsiella tarda* isolated from channel catfish, *Yersinia ruckeri* and *Pseudomonas fluorescens*, isolated from rainbow trout, and *Escherichia coli* (25922), which was obtained from American Type Culture Collection (Rockville, MD, USA). All of these organisms were isolated from clinically affected fish, with the exception of *E. coli* (25922), which was and used as a quality control organism to demonstrate growth and appropriate levels of inhibition.

Four replicate serial dilutions of each drug and drug combination were made in 96-well, polystyrene, micro-titer plates (Becton Dickinson, Lincoln Park, NJ, USA). The dilutions

were made from 128 to 0.25 μg/mL, of total drug, in Mueller–Hinton broth (Difco, Detroit, MI, USA). Two wells in each replicate were also prepared as controls and contained drug-free broth. Each well contained a volume of 100 μL prior to inoculation. Each of the above organisms was diluted in 0.9% sterile saline to yield an approximate concentration of 5.0×10^6 colony-forming units per milliliter. Each set of wells, containing the four replicates of each drug and drug combination, was then inoculated with 5 μL of one of the organisms, with the exception of one of the control wells, which served as the negative control. Each plate was then covered with gas permeable pressure sensitive film (Becton Dickinson, Lincoln Park) and incubated at 21 °C for 18 h. *MIC*s were then determined by visually evaluating growth within each well and reporting the lowest concentration to prevent apparent growth of the organism. Resistance was defined as no visible effect on growth of the organism.

RESULTS

Ormetoprim

The plasma concentration–time profile of ormetoprim after i.p. and oral administration of the sulfadimethoxine/ormetoprim mixture and corresponding pharmacokinetic parameters are shown in Fig. 1 and Table 1, respectively. The data from both the oral and i.p. groups were well described by a one-compartment, open model. The absorption half-life of ormetoprim after i.p. and oral administration was 5.4 and 3.9 h, respectively. The elimination half-life of ormetoprim after i.p. and oral dosing was 7.5 and 3.9 h, respectively. C_{max} was calculated to be 1.6 ± 0.4 μg/mL at 5.7 h for oral administration and 1.2 ± 0.2 μg/mL at 9.1 h for i.p. administration. The *AUC* values for i.p. and oral administration were 31 and 24.3 μg·h/mL, respectively. The oral availability of ormetoprim relative to i.p. administration was 78.5%.

Table 1. Mean pharmacokinetic values for sulfadimethoxine and ormetoprim following i.p. and oral administration of 50 mg/kg sulfadimethoxine and ormetoprim in a 5:1 ratio to hybrid striped bass

	Sulfadimethoxine		Ormetoprim	
	i.p.	Oral	i.p.	Oral
AUC (μg·h/mL)	1141.8	52.1	31.0	24.3
$t_{1/2(elim)}$ (h)	26.0	10.5	7.5	3.9
$t_{1/2(abs)}$ (h)	0.7	0.2	5.4	3.9
C_{max} (μg/mL)*	27.7 ± 2.7	3.2 ± 0.1	1.2 ± 0.2	1.6 ± 0.4
t_{max} (h)	3.6	1.2	9.1	5.7
Relative *F* (%)		4.6		78.5

*Mean ± SD.

Sulfadimethoxine

The plasma concentration–time profile of sulfadimethoxine after i.p. and oral administration of the sulfadimethoxine/ormetoprim mixture and corresponding pharmacokinetic parameters are shown in Fig. 2 and Table 1, respectively. The absorption half-life of sulfadimethoxine after i.p. and oral administration was 0.7 and 0.2 h, respectively. The elimination half-life of sulfadimethoxine after i.p. and oral dosing was 26 and 10.5 h, respectively. C_{max} was calculated to be 3.2 ± 0.1 μg/mL at 1.2 h for oral administration and 27.7 ± 2.7 μg/mL for i.p. administration. The *AUC* values for i.p. and oral administration were 1141.8 and 52.1 μg·h/mL, respectively. The oral availability of sulfadimethoxine relative to i.p. administration was 4.6%.

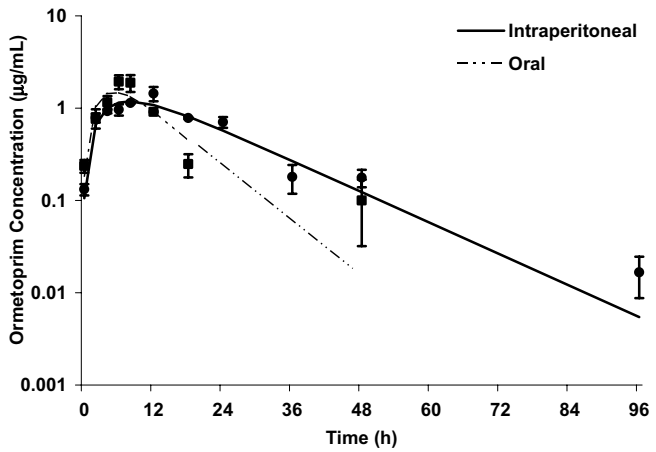

Fig. 1. Mean plasma concentration of ormetoprim in the hybrid striped bass following i.p. and oral administration of sulfadimethoxine and ormetoprim in a 5:1 ratio at a dose of 50 mg/kg body weight. Circles represent mean plasma concentration in three fish, at given time points, and solid line represents best-fit line for i.p. administration. Squares represent mean plasma concentration of three fish at given time points, and dotted line represents best-fit line for oral administration.

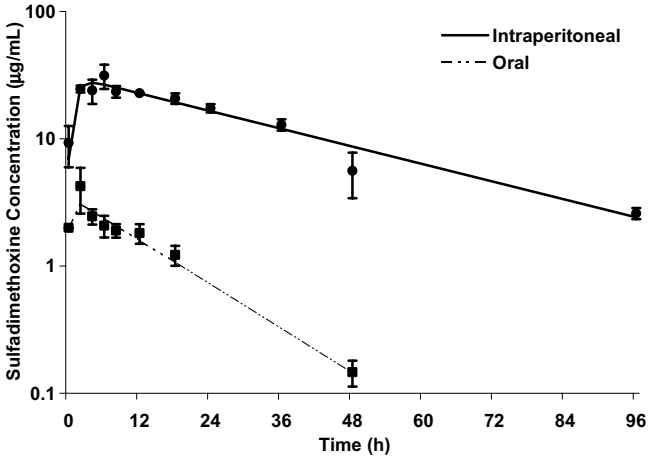

Fig. 2. Mean plasma concentration of sulfadimethoxine in the hybrid striped bass following i.p. and oral administration of sulfadimethoxine and ormetoprim in a 5:1 ratio at a dose of 50 mg/kg body weight. Circles represent mean plasma concentration in three fish, at given time points, and solid line represents best-fit line for i.p. administration. Squares represent mean plasma concentration of three fish, at given time points, and dotted line represents best-fit line for oral administration.

The ratio of the total amount of sulfadimethoxine to ormetoprim found in the serum after oral administration to the hybrid striped bass was 2.14:1 as determined by comparing the *AUC*s for the two drugs. This effectively provides an average of the concentration ratios over time.

Minimum inhibitory concentrations

The *MIC*s for the drugs and drug combinations for the six organisms tested are listed in Table 2. Two organisms, *A. hydrophila* and *P. fluorescens*, were unaffected by the any concentrations of these two drugs individually and to the combinations tested. *Escherichia coli* and *E. tarda* showed greater levels of susceptibility to higher ormetoprim concentrations. *Aeromonas salmonicida* and *Y. ruckeri* had greater susceptibility to the drug combinations, indicating a synergistic effect between these drugs when tested against these organisms.

DISCUSSION

These two drugs, combined in a 5:1 ratio, could not be completely dissolved in our laboratory despite numerous attempts using various solvents, including those previously described in the literature (Droy *et al.*, 1989). This necessitated the use of a suspension, which could not be successfully administered intravenously at our injection site because of concern about embolization of the gills with the suspended drug particles. Injection of these incompletely dissolved mixtures into the sinus venosus invariably resulted in the death of the fish after the appearance of visible hemorrhage from the gills and obvious respiratory distress. Gross necropsy of these animals supported an apparent hemorrhagic anemia based on pallor of the internal organs. We speculate the cause of death in these animals was exsanguination resulting from vascular trauma and occlusion of the micro capillaries of the gill filaments. This problem may not have manifested itself in other studies on other species because of differences in the drug administration site.

As a result of our inability to administer the drug mixture intravenously, we chose to use i.p. administration in the hope that it would closely approximate 100% availability. Intraperitoneal administration does not guarantee 100% availability because there still may be hepatic first pass effects resulting in decreased availability. Additionally, drugs administered via this route can remain sequestered in the peritoneal cavity over prolonged periods reducing apparent availability. This route does, however, eliminate gastrointestinal (GI) effects such as altered absorption and/or metabolism, which may affect the availability of these drugs. However, the calculated mean elimination half-lives of sulfadimethoxine after i.p. and oral administration of the drug combination were 26 and 10.5 h, respectively. This unexpected relationship was likely due to slow absorption of the suspension from the coelomic cavity resulting in an apparent decrease in the rate of elimination. This hypothesis is supported by the slower absorption rate of the drug after i.p. administration (1 μg·mL/h) as compared with the rate after oral administration (3.5 μg·mL/h). This effect may be due in part to an apparent increase in absorptive surface area due to the peristaltic movements of the GI tract. Additionally sulfadimethoxine is more soluble in an acid environment, which could allow for more rapid and greater uptake of the drug from the stomach. The relative oral availability of sulfadimethoxine as compared with i.p. administration (4.6%) indicates that sulfadimethoxine is not well absorbed from the GI tract of hybrid striped bass.

The calculated mean elimination half-lives of ormetoprim after i.p. and oral administration of the drug combination were 7.5 and 3.9 h, respectively. This finding can be explained in the same manner as the sulfadimethoxine half-life findings. The apparent increase in surface area and mixing in the GI tract may enhance absorption while the coelomic cavity may be incapable of rapidly solubilizing the drug suspension contributing to slower absorption. For ormetoprim the absorption rate after i.p. administration (0.1 μg·mL/h) was slower than the absorption rate after oral administration (0.2 μg·mL/h). However the difference, as compared with sulfadimethoxine, is much smaller. The collection of additional data points at longer times postadministration may have helped elucidate the nature of these differences.

Comparisons of our experimental data with that of other kinetic studies in other species are extremely problematic. There is no consistency in the temperatures at which the kinetic studies have been conducted (Kleinow *et al.*, 1992; Kleinow & Lech, 1988; Squibb *et al.*, 1988; Droy *et al.*, 1989, 1990; Michel *et al.*, 1990; Plakas *et al.*, 1990; Walisser *et al.*, 1990). Differences in temperature have significant impacts on absorption and excretion kinetics even within the same species (Borgan *et al.*, 1981). Attempting to compare kinetic studies conducted in different species, at different temperatures is extremely convoluted. Additionally, our study utilized the coelomic cavity as a site of

Table 2. Minimum inhibitory concentrations of sulfadimethoxine (SDM), ormetoprim (OMP), and the two drugs in a 5:1 and 2.14:1 combination, against selected organisms. Number in bold is concentration of the drug combination. Numbers in parentheses are the concentrations of the individual drug components. All concentrations are given in μg/mL. Readings were based on a lack of visible growth

	100% SDM	5:1 SDM:OMP	2.14:1 SDM:OMP	100% OMP
Aeromonas hydrophila	**>128**	**>128** (106.67:21.37)	**>128** (87.04:40.96)	**>128**
Pseudomonas fluorescens	**>128**	**>128** (106.67:21.37)	**>128** (87.04:40.96)	**>128**
Aeromonas salmonicida	**>128**	**8** (6.67:1.33)	**8** (5.45:2.55)	**64**
Yersinia ruckeri	**>128**	**8** (6.67:1.33)	**2** (1.36:0.64)	**2**
Edwardsiella tarda	**>128**	**1** (0.83:0.17)	**0.5** (0.34:0.16)	**0.25**
Escherichia coli	**>128**	**8** (6.67:1.33)	**4** (2.73:1.27)	**1**

administration as opposed to the i.v. route, which further limits the value of any such comparisons.

The maximum concentrations of both sulfadimethoxine (3.2 μg/mL) and ormetoprim (1.6 μg/mL) after a single combined oral dose (50 mg/kg total drug, ratio 5:1) fall within the 4–20 μg/mL *MIC* reported for susceptible strains of *A. salmonicida*, a well-established fish pathogen (Bullock *et al.*, 1974). However, the 5:1 ratio of these two drugs, as administered, does not represent the actual drug ratio found in the plasma of animals. In actuality, due to differential absorption and elimination, this drug combination does not exist in a constant ratio within the animal. This makes *in vitro* determination of the effective concentrations for a drug combination more problematic. It may be more appropriate to use the ratio of the total amounts of the drugs actually absorbed, as expressed by the areas under the curves, than the 5:1 ratio for determining the *MIC*s. By using the ratio of the *AUC*s for the route of administration you would more closely approximate the average ratio of the drugs within the animal. In this case the ratio of the *AUC*s for oral administration was 2.14:1 (sulfadimethoxine-to-ormetoprim). The importance of this issue is well illustrated by the *MIC* data we report for *Y. ruckeri* and *E. coli*. For these two organisms the *MIC*s determined using a 5:1 ratio of the drugs are higher than those calculated using the 2.14:1 ratio obtained from the ratio of the area under the curves. From our experimental data the plasma concentrations of the drugs appear to be high enough to inhibit *Y. ruckeri* and the *E. coli* growth if the *MIC* is based on the 2.14:1 ratio but not the 5:1 drug ratio.

The issue of changing ratios of drug concentrations due to differential absorption and excretion rates also affects the determination of appropriate re-dosing intervals. If the ratio of the *AUC*s is taken into account when determining the *MIC*s for various organisms then the re-dosing interval necessary to achieve and maintain adequate serum concentrations to effectively treat the animal is likely to differ. In this instance, because the 2.14:1 ratio was effective at inhibiting the growth of *Y. ruckeri* and *E. coli* while the 5:1 ratio of the two drugs was not, it is likely that a longer dosing interval would be possible than would have otherwise been determined.

Based on *MIC* data alone, a single oral dose of sulfadimethoxine and ormetoprim (50 mg/kg total drug, ratio 5:1) appears to result in adequate plasma concentrations to inhibit *Y. ruckeri*, *E. tarda*, and *E. coli*. Multiple dose administration should be evaluated to determine if adequate treatment regimes can be developed for hybrid striped bass using this drug combination. Additional work is also needed to evaluate whether an algorithm can be established that is capable of predicting the changing drug ratios and concentrations for this drug combination within the animal. This would allow better determination of effective treatment protocols with this drug combination.

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