Pharmacokinetic and Depletion Studies of Sarafloxacin after Oral Administration to Eel (*Anguilla anguilla*)

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ABSTRACT. The pharmacokinetics of sarafloxacin applied by oral gavage at a dose of 15 mg/kg b.w. was studied in eel (*Anguilla anguilla*) at water temperature of 24°C. Sarafloxacin levels were determined using high performance liquid chromatography with a quantitation limit of 0.07 μg/m*l* or gram. The time to peak plasma concentration, T_{max}, was 12 hr and peak concentration, C_{max}, was 2.64 μg/m*l*. The absorption rate constant (k_a) was 0.23 hr^{-1} (r=0.996). The drug disposition curve after T_{max} was fitted to a two-compartment open model. The distribution rate constant (α) was 0.085 hr^{-1} (r=0.972), and the half-life ($t_{1/2\alpha}$) was 8.15 hr. The elimination rate constant (β) was 0.023 hr^{-1} (r=0.909), and the half-life ($t_{1/2\beta}$) was 30.13 hr. The estimated area under the curve, AUC, was 56.7 μg.hr/m*l*. The peak concentrations of drug in liver, kidney, muscle, and skin were 13.39 (12 hr), 5.53 (12 hr), 1.82 (24 hr), and 0.78 μg/g (40 hr), respectively. The time for sarafloxacin mean levels to fall below detectable limits in the plasma, muscle, and skin were 7 days but for the liver and kidney were 14 days.—KEY WORDS: eel, fish, HPLC, pharmacokinetics, sarafloxacin.

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Sarafloxacin is a fluorinated quinolone that has a broad-spectrum of antibacterial activity, especially against Gram-negative bacteria, and can be used for the treatment of a variety of important infectious diseases in cultured fish, such as *Aeromonas salmonicida* [8], *Edwardsiella* spp. [7, 19], and *Vibrio* spp. [3].

Pharmacokinetic studies are important for defining the dosage regimens of antibacterial agents. However, the knowledge of pharmacokinetics and residues of antimicrobial agents in fish is still very limited. It has been recognized that the pharmacokinetics of drugs in fish depend on several factors, such as species [5, 16], water temperature, pH [18], and salinity [6, 17]. The pharmacokinetics of various drugs in Atlantic salmon (*Salmo salar*) [10–14], channel catfish (*Ictalurus punctatus*) [4], cod (*Gadus morhua*), rainbow trout (*Oncorhynchus mykiss*), and turbot (*Scophthalmus maximus*) [14] have been studied. However, no information on the pharmacokinetic properties of sarafloxacin in eel (*Anguilla anguilla*) has been reported. This study was carried out to examine the pharmacokinetic properties and tissue residues of sarafloxacin applied by oral gavage in eel kept at 24°C. High performance liquid chromatography (HPLC) was used to detect sarafloxacin in plasma and tissues.

MATERIALS AND METHODS

Fish: Eels (*Anguilla anguilla*) weighing 146 ± 3 g, were obtained from the Taishi Marine Laboratory of the Taiwan Fisheries Research Institute. The fish were kept in freshwater in a fiberglass tank of 30-liter capacity (5 fishes/tank) and fed with commercial fish feed. The fish were acclimatized for 2 weeks and starved 48 hr before the experiment. The water temperature was 24 ± 0.7°C.

Chemicals: Sarafloxacin was obtained from Abbott Lab., North Chicago, Illinois, U.S.A. Other chemicals were analytical grade or HPLC grade.

Oral administration and sampling: The solution of sarafloxacin was prepared by dissolving in 0.1 N NaOH (pH 10.5). The dosage was 15 mg/kg b.w. After the oral gavage, samples were taken on hours 1, 2, 4, 8, 12, 16, 20, 24, 28, 32, 36, 40, 44, 48, 72, 96, 168, 240, 336, and 504. Five fishes were sacrificed at each time point. Samples of blood, kidney, liver, muscle, and skin were collected from each fish. Blood samples were taken by caudal venepuncture and heparinized vacutainers. After centrifugation, the plasma was removed and kept in plastic vials. All samples were kept frozen at −20°C until analyzed.

Sample preparation: The extraction and clean-up procedures for sarafloxacin from fish tissue are illustrated in Fig. 1. The kidney, liver (0.5 g each), and muscle and skin (3.0 g each) samples were homogenized in 50-m*l* centrifuge tubes, with 5 m*l* extraction solvent (distilled water: acetonitrile=1:4), for 30 seconds using a high-speed blender (Yamato Scientific Co., Ltd, Tokyo, Japan). The mixture was centrifuged (5 min, 1,800 g, Kubota Corp, Tokyo, Japan) and supernatant was transferred to a separatory funnel. The above procedure was repeated again on the remaining solid residue. To the combined extract, 200 μ*l* 1 N NaOH and 20 m*l* ethyl acetate-hexane (3:2) were added. The mixture in the separatory funnel was blended thoroughly, the upper layer of organic solvents was discarded, and the aqueous solution was collected. The aqueous tissue extract and the plasma (250 μ*l*) samples were cleaned up by solid-phase extraction on a column of the Bond Elute type, size 1 m*l*, with C_2 sorbent material (J. T.

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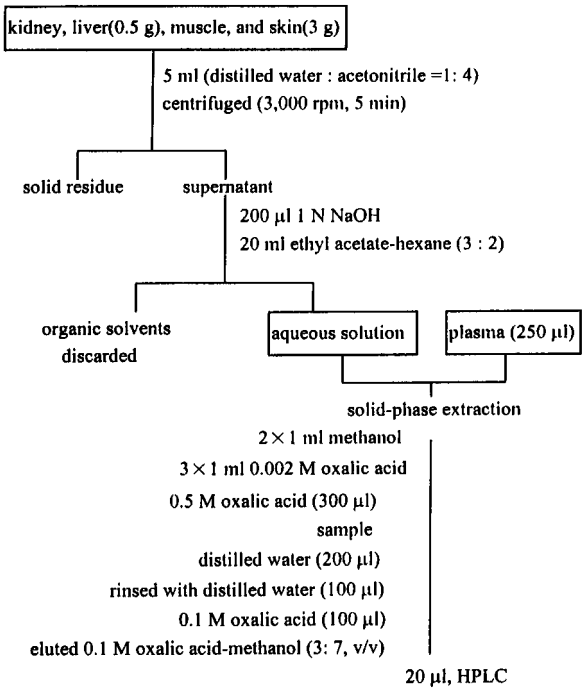

Fig. 1. Extraction and clean-up procedure for sarafloxacin from fish tissue and plasma.

Baker Inc., Phillipsburg, NJ, U.S.A.) [20]. The extraction column was first conditioned with 2 × 1 m*l* methanol and 3 × 1 m*l* 2 mM oxalic acid. The column then was loaded, in the following sequence, with 0.5 M oxalic acid (300 μl), sample, distilled water (200 μl), all being sucked through at 4 psi. Then, the column was rinsed with distilled water (100 μl) 0.1 M oxalic acid (100 μl). Finally, sarafloxacin was eluted from the column with an equal volume as sample of 0.1 M oxalic acid-methanol (3:7, v/v). Twenty μl of the eluent were injected into the HPLC system for determination of sarafloxacin.

Method of sarafloxacin analysis: The concentration of sarafloxacin in plasma and tissue samples was determined by HPLC. The HPLC system consisted of a degasser (Uniflows Co., Ltd. Tokyo, Japan), a constant-flow pump (Perkin Elmer series 200 LC pump, Norwalk, CT, U.S.A.) and a UV detector (Perkin Elmer LC 295 UV/VIS detector, Norwalk, CT, U.S.A.) operated at 278 nm. The separation process was performed on LiChrospher RP-18 columns (125 × 4 mm I. D., 5 μE. Merck, Darmstadt, F. R. Germany) connected to a guard column (4 × 4 mm I. D., RP-18, 5 μE. Merck, Darmstadt, F.R. Germany) with 0.01 M oxalic acid-acetonitrile-methanol-tetrahydrofuran (7.5:1.5:0.5:0.5, pH=3.0) as the mobile phase at a flow-rate of 1.2 m*l*/min at room temperature. An integrator (Perkin Elmer PE Nelson model 1022, Norwalk, CT, U.S.A.) was used to collect the chromatographic data.

Pharmacokinetic analysis: Plasma sarafloxacin concentration-time data were plotted as the drug concentration versus time from drug administration. The peak concentration (C_{max}) and the corresponding peak time (T_{max}) were read from the fitted concentration vs time curve, respectively. The SAS program (SAS Institute Inc., Cary, NC, U.S.A.) was used to calculate data. The residual method was applied to calculate the absorption rate constant (k_a). A least-squares regression was used for analysis of the plasma concentration curve beyond T_{max} to resolve the following equation: $C_t = Axe^{(-\alpha t)} + Bxe^{(-\beta t)}$, where C_t is the plasma concentration, t is the time, and α and β are values related to the slopes of distribution and elimination phases, respectively, of the biexponential disposition curve of sarafloxacin. A and B are the zero-time plasma drug concentrations. The distribution half-life ($t_{1/2\alpha}$) and elimination half-life ($t_{1/2\beta}$) were calculated from $t_{1/2\alpha \text{ or } \beta} = (0.693)/\alpha$ or β. The estimated area under the mean concentration-time curve (AUC) and its variance (S^2) were calculated using the trapezoid rule as Bailer's method [1].

$AUC = \sum c_{(i)} Y_{(i)}$

$Y_{(i)}$ = mean of the plasma concentration at time t_i; i =1, 2, 4,....96

$c_{(l)} = \Delta_2/2$; $c_{(i)} = (\Delta_i + \Delta_{i+1})/2$; (i = 2, 4,*..72); $c_{(96)} = \Delta_{96}/2$; $\Delta_i = t_{(i)} - t_{(i-l)}$; An estimate of the variance associated with AUC is

$S^2 = \sum c_i^2 (S_i^2/n)$; i= 1, 2, 4,.....96; n=5

RESULTS

Recovery and quantitation limit: Figure 2 presents chromatograms of blank samples of plasma, kidney, liver, muscle, and skin spiked with 1 ppm sarafloxacin. The retention time of sarafloxacin was within the range of 3.6~3.9 min. The standard curve of the spiked sample was found to be linear over the range of 0.07 to 25 μg/m*l* or gram. A good correlation was obtained between concentration of sarafloxacin and peak area (r=0.999). The recoveries and quantitation limit (s/n>10) of sarafloxacin are shown in Table 1. The recoveries of sarafloxacin from plasma, kidney, liver, muscle, and skin based on peak area were 78.2–91.1%, 78–87.4%, 75.7–86.3%, 73.4–79.7%, and 74–87.7%, respectively. The quantitation limit of sarafloxacin in plasma and in tissues was 0.07 μg/m*l* or gram.

Pharmacokinetics: Following single oral administration at a dose of 15 mg/kg b.w., the graphs of sarafloxacin concentration vs time of the plasma, kidney, liver, muscle, and skin of eel are shown in Fig. 3 and Fig. 4. Pharmacokinetic parameters are noted in Table 2. This plasma concentration time data of sarafloxacin corresponded to a two-compartment open model ($C_t = 6.854 \times e^{(-0.085t)} + 0.625 \times e^{(-0.023t)}$.

The C_{max} in the samples of kidney, liver, muscle, and skin were 5.53, 13.39, 1.82, and 0.78 μg/g, respectively. The T_{max} of kidney, liver, muscle, and skin were 12, 12, 24, and 40 hr, respectively. At their highest levels, the concentrations of sarafloxacin in tissues were observed in the following order: liver>kidney>muscle>skin. The time for sarafloxacin tissue mean levels to fall below the

detectable limit in the plasma, muscle, and skin were 7 days but for the kidney and liver were 14 days.

DISCUSSION

The pharmacokinetics of sarafloxacin was studied in eel kept at 24°C after a single oral dose of 15 mg/kg b.w. The drug concentrations in the samples were determined by an HPLC system. It was found that sarafloxacin had maximum optical absorption at 278 nm. An acidified eluting solution was used to gain a better peak shape in the chromatograms. In this study, results showed high recovery rates and linearity between drug concentration and peak area, indicating that this HPLC method was suitable for the measurement of sarafloxacin in samples from eel.

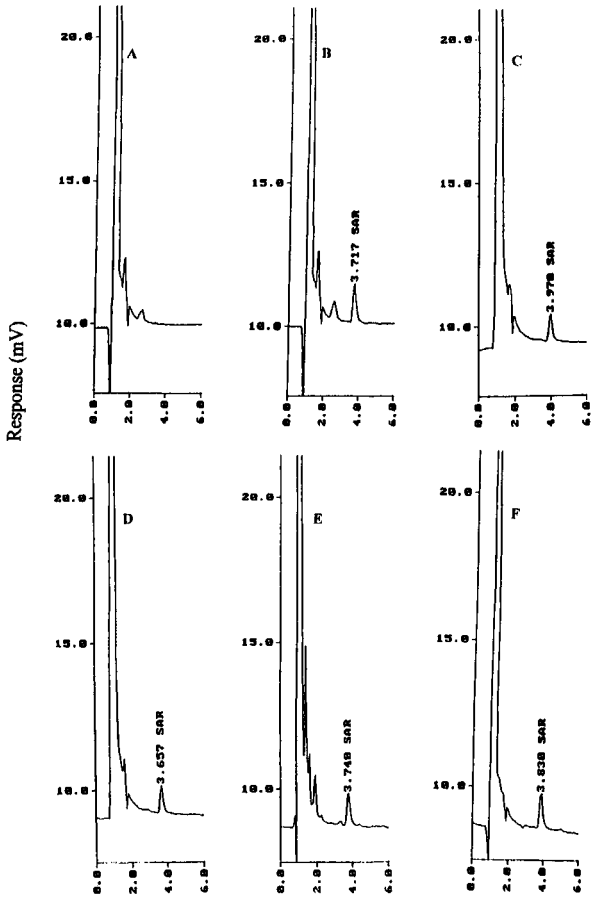

Fig. 2. Chromatograms of extracts from eel plasma and tissues spiked with sarafloxacin 1 μg/m*l* or gram. A: blank (plasma), B: plasma, C: kidney, D: liver, E: muscle, F: skin.

The present data showed that the pharmacokinetic parameters for sarafloxacin in eel could be expressed by a two-compartment open model after a single oral administration of 15 mg/kg b.w. The C_{max} in plasma (2.64 ± 0.42 μg/m*l*), kidney (5.53 ± 0.52 μg/g), and liver (13.39 ± 1.65 μg/g) were achieved at t=12 hr, and C_{max} in muscle was 1.18 ± 0.17 μg/g at t=24 hr. It has been reported that the T_{max} was 12 hr in plasma at 12°C using a single oral dose of 10 mg/kg b.w. for Atlantic salmon [12]. These T_{max} values correspond to the liposolubility of the drug. In our study, the maximum levels of sarafloxacin in eel occurred in the following order: liver>kidney>plasma>muscle>skin. This trend of disposition was also seen in the Atlantic salmon medicated orally with sarafloxacin [13, 14].

Pharmacokinetic data may be used to establish a clinical regimen by which plasma levels of a drug can be maintained above the minimum inhibitory concentration (MIC) by repeated dosages. The MICs of sarafloxacin against *A. salmonicida*, *V. salmonicida*, *V. anguillarum*, and *Yersinia ruckeri* have been reported to range from <0.0025 to 0.3 μg/m*l* for quinolone-susceptible strains [2, 9, 10]. The MICs of sarafloxacin against *V*. spp. isolated from fresh and sea water fish were 0.015–3.12 μg/m*l* and 0.0015–1.56 μg/m*l* [21] respectively. In the present study, the C_{max} were approximately at the upper limit of the MIC-ranges reported. It is proposed that a dose of 15 mg/kg b.w. would be acceptable for treatment of eel with bacterial infections.

Cultured eel is an important resource of fish in Asia. The drug residues in tissues should be monitored to ensure safety. In this study, the time points for mean sarafloxacin levels to fall below detectable limit in the plasma, muscle, and skin were 7 days but those for kidney and liver were 14 days.

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Table 1. Recovery of sarafloxacin from spiked samples of eel tissues and plasma

Concentration	Recovery (%) ± S.D. (n=3)				
μg/m*l*(g)	Muscle	Skin	Liver	Kidney	Plasma
25	75.2 ± 4.3	80.2 ± 3.3	78.3 ± 2.9	81.5 ± 2.5	80.0 ± 3.9
5	73.4 ± 5.2	85.8 ± 4.5	81.6 ± 2.7	87.4 ± 3.8	80.7 ± 2.9
1.25	78.0 ± 6.0	87.7 ± 3.5	86.3 ± 4.4	82.1 ± 4.4	90.6 ± 3.8
0.25	79.7 ± 4.0	86.7 ± 3.2	81.9 ± 2.9	85.7 ± 6.3	91.1 ± 2.1
0.1	77.5 ± 2.4	83.0 ± 3.1	76.6 ± 3.3	82.6 ± 1.3	83.6 ± 1.8
0.07	74.0 ± 2.3	74.0 ± 1.9	75.7 ± 3.1	78.0 ± 2.7	78.2 ± 2.4
0.05	–[a)]	–	–	–	–

a) Undetectable.

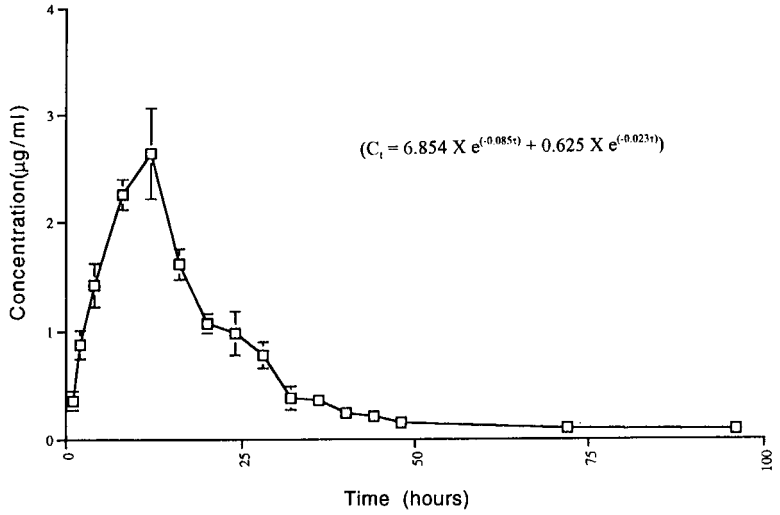

Fig. 3. Plasma concentration (mean ± SD, n=5) of sarafloxacin in eel (*Anguilla anguilla*) after single oral gavage of 15 mg/kg body weight at 24°C.

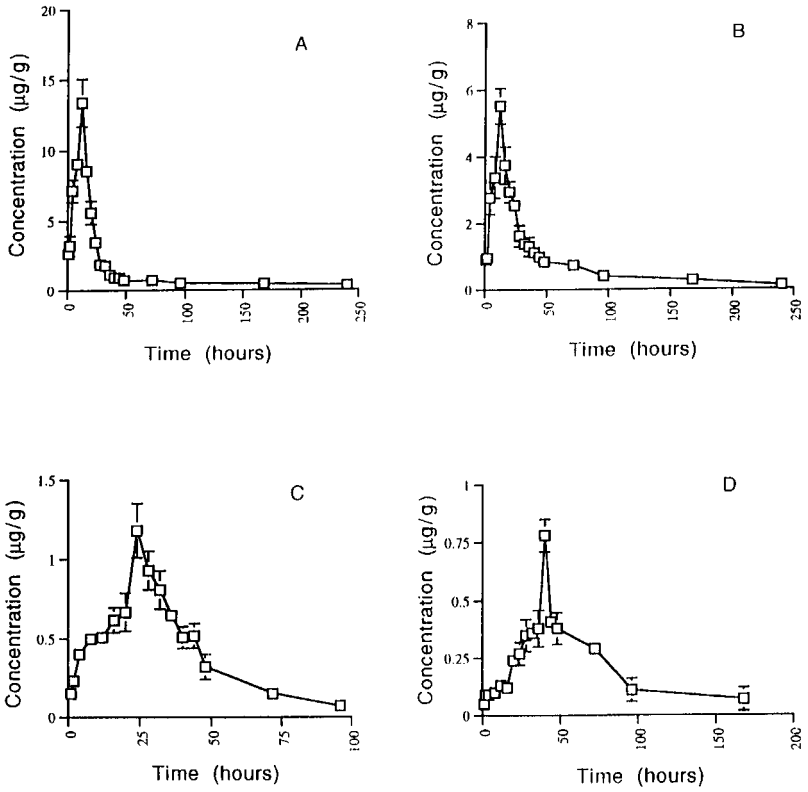

Fig. 4. Tissue concentration (mean ± SD, n=5) of sarafloxacin in eel (*Anguilla anguilla*) after single oral gavage of 15 mg/kg body weight at 24°C. A: liver; B: kidney; C: muscle; D: skin.

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Table 2. Pharmacokinetic parameters describing the disposition of sarafloxacin in eel (*Anguilla anguilla*) at 24°C after single oral administration (15 mg/kg b.w.) (n=5; ± S.D.)

Kinetic parameters	Units	Values
Absorption rate constant (k_a)	hr^{-1}	0.23
half-life	hr	3.01
Peak plasma concentration, C_{max}	μg/m*l*	2.64 ± 0.42
Time to peak plasma concentration, T_{max}	hr	12
Distribution rate constant (α)	hr^{-1}	0.085
half-life($t_{1/2\alpha}$)	hr	8.15
Elimination rate constant(β)	hr^{-1}	0.023
half-life($_{1/2\beta}$)	hr	30.13
Area under curve, AUC	μg.hr/m*l*	56.7 ± 1.70

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