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Effects of Temperature on the Pharmacokinetics of Ciprofloxacin in the Cultured Black Rockfish (*Sebastes schlegeli*) and Olive Flounders (*Paralichthys olivaceus*)

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Temperature-dependent pharmacokinetics of ciprofloxacin (CIP) was studied in the cultured olive flounders, *Paralichthys olivaceus*, and black rockfish, *Sebastes schlegeli*, using high performance liquid chromatography (HPLC) originally developed for quinolone determination from livestock. Pharmacokinetics of CIP was apparently affected by ambient water temperature. In a two-compartment model for flounders after oral dosage of 20 mg/kg, K_{01} at 13℃ and 23℃ were 4.18 and 1.20/hr, respectively. The K_{10}, T_{max} and C_{max} at 13℃ were 5.574/hr, 14.37 μg/mL and 3.15 μg/mL, respectively. The corresponding values at 23℃ were 12.84/hr, 15.39 μg/mL and 6.38 μg/mL, respectively. The AUC, $T_{1/2}$ (α) and $T_{1/2}$ (β) were 278.23 μg·hr/mL, 0.24 hr and 47.02 hr at 13℃ and 317.81 μg·hr/mL, 0.30 hrs and 60.78 hrs at 23℃ for the flounder, respectively. Similar CIP pharmacokinetics were revealed in the black rockfish after oral dosage of 20 mg/kg under the two water temperature regimes. These pharmacokinetical results have some implication in the optimal usage of recently introduced antibacterials in the farmed fish, which were primarily adapted for poultry and mammalian species.

Key words: Ciprofloxacin, Pharmacokinetics, Temperature, HPLC, Olive flounder, Black rockfish

Introduction

Ciprofloxacin (CIP), a fluoroquinolone antibacterial, first developed as a human medicine, gained worldwide acceptance for the oral and intravenous treatments of a range of bacterial infections in veterinary (Takemura and Hayakawa, 2001). CIP has an antibacterial activity by primarily inhibiting DNA gyrase, an enzyme that is critical to bacterial DNA replication, transcription, repair, and recombination. CIP has broad-spectrum activities against many gram-positive and gram-negative bacteria and atypical pathogens, such as *Mycoplasma* spp. (Vance et al., 1990; Hooper and Wolfson, 1993; Kreuzer, 1998). Quinolones are currently the most commonly used antibacterials in cultured fish (Grave et al., 1996; Samuelsen and Ervik, 1999). However, pharmacokinetical informations on CIP administered to fish are still lacking.

In Korea, some fish farmers have used CIP for the control of the bacteria-infected cultured black rockfish (*Sebastes schlegeli*) and olive flounders (*Paralichthys olivaceus*) which have been principal marine finfish cultured in the country. CIP can provide aquaculturists with a good way of keeping the cultured fish from bacterial diseases in their farms, but it needs an appropriate prescription prior to use. The use of CIP may leave residue that poses potential health hazards to consumers of the product when it is used over optimal dosage. As with most antibiotics, prolonged exposure of bacterial population to ineffective dosage of CIP can result in deve-

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lopment of resistant pathogens. Thus, it is extremely important to get pharmacokinetical information of CIP before it is approved to be used for the control of the bacterial disease in the fish farms. It also has some significant implication in the legislation on CIP usage for other fish species because pharmacokinetics of veterinary CIP reported has predominantly focused upon poultry and mammalian species (Intorre et al., 1997; Ovando et al., 1999; Cester and Toutain, 1997).

In the present study, we investigated the pharmacokinetics of CIP orally administrated in the cultured rockfish and flounders at two different temperature regimes. Temperature-dependent elimination time of the antibacterial was determined to define appropriate use of the antibacterial in the fish farm.

Materials and Methods

Fish, antibacterial dosage and sampling

Cultured olive flounders, *Paralichthys olivaceus*, weighing 700 ± 50 g, and black rockfish, *Sebastes schlegeli*, weighing 500 ± 50 g, without previous exposure to any antibacterials, were maintained at a temperature of 15℃ for a week for an acclimation in captivity. A month acclimation has been additionally made after 28 fish were separated into four aquaria (7 fish per aquarium) set in two different temperature regimes; two aquaria in 13 ± 1.5℃ regime and another two in 23 ± 1.5℃. All the fish were, then, orally administered with CIP (Cheilchedang Co., Korea) at dosage of 20 mg/kg. Blood (0.3~0.4 mL) was serially taken from caudal vessel of the fish anesthetized with MS-222 (Sigma, USA) at 1, 5, 10, 15, 24, 35, 50, 72, 120, 168, 216, 312, 480, 720 and 840 hr using heparin-treated syringes.

Extraction of CIP residue

Extraction of CIP in the fish was based on the methods developed for fluoroquinolones in poultry (Belal et al., 1999; Yorke and Froc, 2000; Posyniak et al., 2001). In brief, the fish blood samples of 200 μL taken were quickly eluted with 200 μL of 0.1 N-NaOH solution. The samples were stirred with a glass rod, homogenized, and allowed to stand at room temperature (25℃) for 10 min. An amount of 200 mL chloroform was, then, added to the homogenate. The samples were shaken for 5 min and centrifuged at 10,000 rpm for 20 min at 4℃. The supernatant was filtered (Sartorius AG, Germany, 0.45 μm). The filtered sample was either stored at −18℃ or immediately analyzed by HPLC.

HPLC analysis

Analysis of the samples was performed with a HPLC system (Hitachi L-6200) equipped with an UV detector (Hitachi L-4250) set at 287 nm absorbance and 0.002 A.U.F.S sensitivity. Sample (20 μL each) was separated using a reverse phase with a COSMOSIL column (C18, 5 μm, 150×4.6 mm i.d.). The mobile phase was 8 mL of filtered phosphoric acid:triethylamine (1:1, v/v, per liter) in water: acetonitrile:methanol (700:200:100, v/v/v) at a flow rate of 1.0 mL/min. Peak areas of CIP external standard at 0.095, 0.19, 0.39, 0.78, 1.56, 3.12, 6.25 and 12.5 ppm were determined for a calibration curve. CIP concentration of sample was quantified by peak area relative to external standard.

Results

Calibration curve and ciprofloxacin concentrations in samples

A calibration curve representing standard CIP between 0.095 and 12.5 ppm injected into HPLC set at 287 nm absorbance was linearly expressed as $Y = 239528X + 8009.9$ ($r^2 = 0.9993$). Recovery rates of internal CIP (0.01, 0.1, 1.0 and 10 ppm) added to liver, muscle, spleen, kidney, and blood of the black rockfish ranged 80.26 ± 3.80 to 93.38 ± 2.21% when all the samples were extracted in the 0.1 N-NaOH solution. Similar results were achieved in the samples from olive flounder with recovery rates for CIP ranging from 79.94 ± 3.82 to 95.24 ± 2.29% (Table 1).

Blood ciprofloxacin levels at different water temperatures

The residual CIP levels in black rockfish and olive flounder orally administered with CIP 20 mg/kg at two different temperatures, 13 and 23℃, are shown in Fig. 1. Water temperature clearly affected absorption and persistence of CIP in the fish blood. Olive flounder maintained at 13℃ had 2.0521 (± 0.37500) μg CIP/mL blood at 1 hr after oral administration. The blood level of CIP increased to 3.0324

Table 1. Recovery of ciprofloxacin from tissues of olive flounder and black rockfish

Tissue	Fortification level (μg/g)	Recovery (%)	
		Olive flounder	Black rockfish
Liver	10.0	90.25 ± 2.18	85.94 ± 5.02
	1.0	87.97 ± 2.51	82.68 ± 1.73
	10.0	86.34 ± 3.00	85.40 ± 2.70
	0.01	93.38 ± 2.21	95.24 ± 2.29
Muscle	10.0	89.08 ± 1.18	84.11 ± 1.27
	1.0	84.89 ± 1.96	81.86 ± 1.36
	10.0	83.25 ± 1.96	81.00 ± 1.69
	0.01	86.85 ± 5.54	82.45 ± 6.22
Spleen	10.0	84.66 ± 2.28	82.91 ± 3.89
	1.0	83.59 ± 1.75	82.11 ± 6.01
	10.0	87.90 ± 4.14	80.30 ± 2.13
	0.01	91.38 ± 5.49	86.80 ± 2.47
Kidney	10.0	86.03 ± 3.16	85.80 ± 1.91
	1.0	81.95 ± 3.80	90.43 ± 4.38
	10.0	80.26 ± 3.80	81.01 ± 2.37
	0.01	82.74 ± 3.90	96.01 ± 7.56
Blood	10.0	83.40 ± 3.09	82.76 ± 2.29
	1.0	81.29 ± 2.05	79.94 ± 3.82
	10.0	82.80 ± 2.59	80.39 ± 3.96
	0.01	91.97 ± 2.04	83.92 ± 6.58

(± 0.6324) μg/mL at hour 10 and 3.2194 (± 0.2587) μg/mL at hour 15 before it gradually decreased to 0.0571 (± 0.0055) μg/mL at day 20 and close to zero (infinitesimal concentrations only in 3 of 7 fish) at day 30. A total degradation of the antibacterial was noted at day 35. CIP concentration of the fish acclimated to 23℃ was 3.9378 ± 0.1345 μg/mL blood 1 hr after administration. This increased to 6.2330 (± 0.1442) μg/mL by 10 hours and to 6.4792 (± 0.1040) μg/mL by 15 hr. The elevated blood CIP persisted through a day, 5.2130 (± 0.0927) μg/mL by 35 hr. As in the fish acclimated to lower temperature, the blood residual CIP was persistent with the residual detection of 0.0248 (± 0.0012) μg/mL by day 30. At day 35, no CIP was detected in the blood of the fish (Fig. 1A).

Temperature effects on CIP absorption and persistency in black rockfish blood are illustrated in Fig. 1B. The blood CIP of the fish started to increase from 1 hr after dosage and reached maximum, 4.5425 (± 0.1576) μg/mL by hour 24 via 4.0957 (± 0.2364) μg/mL by hour 10 at 13℃. Thereafter, the residual concentration decreased gradually and continuously to 2.8631 (± 0.2366) μg/mL by hour 50 and 1.6354 (± 0.0716) μg/mL by hour 72. As in the case of olive flounder, higher water temperature, 23℃, supported absorption and persistency of CIP in the fish blood. The antibacterial level became 8.0316 (± 0.3080) by 10 h and maximized itself by showing 8.2634 (± 0.1113) μg/mL by 24 h. These levels at 23℃ are two-fold higher than those at 13℃. Once maximized, it started to decrease to 3.5070 (± 0.1065) μg/mL by hour 35, 2.811 (± 0.1362) μg/mL by 3 d, and 0.0015 ± 0.0001 μg/mL by 30 d.

Pharmacokinetics of ciprofloxacin

Based on the method by Kim et al. (1998), pharmacokinetics of CIP in the fish acclimated to two different water temperatures were obtained by analyzing data from blood CIP levels (Table 2). In a two-compartment model, constant K_{01} for absorption of CIP at 13 and 23℃ were 4.18, 1.20/hr, respectively, for olive flounder. In a mean while, the constant K 10 for elimination CIP at 13 and 23℃ were 5.57 and

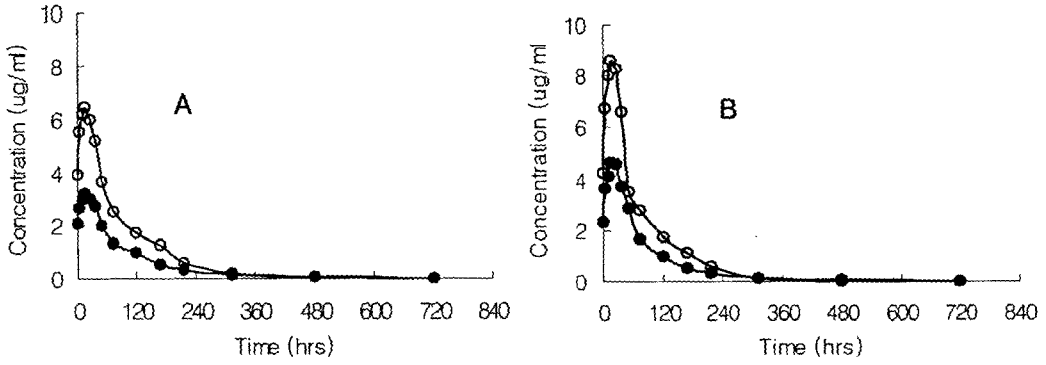

Fig. 1. Blood levels of ciprofloxacin in olive flounder (A) and black rockfish (B) after oral CIP dosage of 20 mg/kg at 13 ± 1.5℃ (—●—●—) and 23 ± 1.5℃ (—○—○—), where higher water temperature induced higher frequencies in the residual CIP levels, affecting pharmacokinetic properties of the antibacterial in the both fish.

Table 2. Effects of water temperature on pharmacokinetic parameters of ciprofloxacin in olive flounder and black rockfish plasma

Parameters (unit)	Olive flounder		Black rockfish	
	13 ± 1.5℃	23 ± 1.5℃	13 ± 1.5℃	23 ± 1.5℃
AUC (μg·hr/mL)	278.23	317.81	350.00	325.89
K_{01} (1/hr)	4.18	1.20	4.24	1.20
K_{10} (1/hr)	5.57	12.84	6.33	14.11
$T_{1/2}$ (α) (hr)	0.24	0.30	0.24	0.28
$T_{1/2}$ (β) (hr)	47.02	60.78	37.75	54.21
T_{max} (hr)	14.37	15.39	16.15	19.39
C_{max} (μg/mL)	3.15	6.38	4.54	8.88

Abbreviations: AUC, area under serum concentration-time curve; K_{01} and K_{10}, distribution rate constants of central and peripheral compartments; $T_{1/2}$ (α), absorption half-life; $T_{1/2}$ (β), elimination half-life; T_{max}, time for maximum concentration; C_{max}, maximum concentration.

12.84/hr, respectively. T_{max}, time for maximum concentration, at 13 and 15℃ were 14.37 and 15.39 hr and their representing CIP concentrations, C_{max}, were 3.15 and 6.38 μL/mL, respectively. The areas under blood concentration-time curve, AUC, were 278.23 μg·hr/mL at 13℃ and 317.81 μg·hr/mL at 23℃. The $T_{1/2}$ (α), the absorption half-life, at 13 and 23℃ were 0.24 and 0.30 hr, while the elimination half-life, $T_{1/2}$ (β), at the two temperatures were 47.02 and 60.78 hr, respectively.

Pharmacokinetic parameters for CIP following oral dosage to black rockfish were also determined. K_{01} for CIP at 13 and 23℃ were 4.24 and 1.20/hr, while K_{10} at the temperatures were 6.33 and 14.11/hr, respectively. T_{max}, C_{max}, AUC, $T_{1/2}$ (α), and $T_{1/2}$ (β) at each temperature were 16.15 and 19.39 hr, 4.54 and 8.88 μg/mL, 350.00 and 325.89 μg·hr/mL, 0.24 and 0.28 and 37.75 and 54.21 hr, respectively.

Discussion

The fluoroquinolones are long-acting, broad-spectrum, and well-tolerated antibacterials with an antimicrobiological action by inhibiting DNA gyrase, an enzyme required for bacterial DNA replication. CIP, a chemically novel fluoroquinolone developed in Germany, has been used as a therapeutic antibiotic against bacteria responsible for various human infectious diseases such as urinary tract infections (Newson et al., 1986), enteric infections (Dupont et al., 1987) and osteomyelitis (Lesse et al., 1987). With a successful introduction of CIP to livestock farming, its further introduction to aquaculture has gained regional acceptance for oral treatment of diseased fish with a wide range of bacterial infections.

As in the cases of livestock, CIP is believed to provide fish farmers a way of controlling diseases in their cultured fish as long as it is used appropriately. However, limited data are available for optimal dosage for the infected fish on the basis of pharmacokinetics. Determination of residual CIP in fish is in priority to pharmacokinetic study for appropriate drug dosage. Belal et al. (1999) reviewed the application of HPLC to the determination of CIP. Based on their suggestion and other studies (Yorke and Froc, 2000; Posyniak et al., 2001), we determined CIP from the cultured *Paralichthys olivaceus* and *Sebastes schlegeli* orally administered to the drug in two temperature regimes. The frequency against CIP injected was linearly expressed in concentrations 0.095 to 12.5 μg/mL: $Y=239528X+8009.9$ ($r^2=0.9993$). Recovery rates of internal CIP concentrations at 0.01, 0.1, 1.0 and 10 ppm from organs of rockfish and flounders ranged from 80.26 to 93.38% and from 79.94 to 95.24%, respectively. A peak representing sample CIP was clearly noticed at retention time 5.17 min in a chromatogram.

CIP has good absorption, wide distribution in tissues and a broad spectrum of *in vitro* activity against bacterial activities. In human, the antibacterial is rapidly liberated in most body fluids and tissues and quickly attains high concentration, which, thereafter, falls exponentially with time (Bakhouya et al., 1996). Ingested to human body, 60% to 80% of CIP is absorbed, with peak serum concentrations at 1 to 1.5 hrs. Increased doses produce correspondingly higher serum concentrations; for example, doses of 250, 500 and 1,000 mg in adults produce mean peak concentrations of approximately 1.7, 2.3 and 5.9 mg/L, respectively (Bergan et al., 1987). Therefore, the absorption and elimination studies of antibiotics in animals living different environments have been a prerequisite to aquaculture management, offering fish farmers criteria not only for the optimal dosage, but for depuration by keeping their fish from treatment of the antibiotics prior to marketing (Herman et al., 1969; Kasuga et al.,

1984; McCracken et al., 1976; Uno et al., 1993).

Temperature is one of parameters affecting pharmacokinetics of antibacterials in fish (Jacobson, 1989). It affects pharmacokinetics of quinolones with different absorptions in the animal tissues. Oxolinic acid (OA) administrated to yellowtails (20 mg/kg·b.w.) remained 1.2 μg/g tissue 24 hr after the administration at 10℃, while remained 2.5 μg/g by the time at 20℃. Kim et al. (1998) reported that absorption rates of OA in tilapia differed by the different ambient water temperature.

In the present study, we took two temperatures, 13 (±1.5) and 23 (±1.5) ℃, the temperature regime where the two fish are normally encountered in the temperate regions. The temperature difference did affect the half-life of CIP, but not significantly. It only affected the maximal blood CIP concentration with a significance, in which the maximal CIP concentrations at 13 and 23℃ were 3.15 μg/mL and 6.38 μg/mL for flounders and 4.54 μg/mL and 8.88 μg/mL for rockfish, respectively. The results provide the fish farmers with some basic information on the seasonal usage of the antibacterial temperature to the farmed fish. However, considering that antibacterial pharmacokinetics can be affected by the disease stress and physiological status of the fish, further studies are still in need before the drug is effectively and safely used in the two cultured fish species.

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