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Pharmacokinetics and the active metabolite of enrofloxacin in Chinese mitten-handed crab (*Eriocheir sinensis*)

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Abstract

The pharmacokinetics and active metabolite of enrofloxacin were estimated after single intramuscular administration (10.0 or 20.0 mg/kg body weight) to the Chinese mitten-handed crab (*Eriocheir sinensis*) in fresh water at 25.0±1.0 °C. Levels of enrofloxacin and its metabolite ciprofloxacin in the main tissues (hemolymph, hepatopancreas, muscle, ovary and spermary) were simultaneously detected by HPLC. Enrofloxacin concentration–time profiles for the hemolymph in both tests were described by a two-compartment open model with first-order absorption. Distribution half-time ($T_{1/2\alpha}$), elimination half-time ($T_{1/2\beta}$), body clearance (CL/F), mean residence time ($MRT_{0-\infty}$), area under the concentration–time curve from 0 to ∞h ($AUC_{0-\infty}$) and apparent volume of distribution (Vd/F), which derived from the pharmacokinetic model, were 0.427 h, 21.3 h, 0.133 l/h/kg, 60.0 h, 96.9 μg/ml/h and 4.08 l/kg, respectively, at a dose of 10.0 mg/kg body weight, and 0.216 h, 12.3 h, 0.189 l/h/kg, 85.8 h, 187 μg/ml/h and 3.35 l/kg, respectively, at a dose of 20.0 mg/kg body weight. Similarities were found between the hemolymph concentration–time curves of the two tests; for example, instant absorption process followed by the distribution phrase, and a second absorption peak at 6 h post-treatment. After intramuscular administration of 10.0 mg/kg body weight, absorption of enrofloxacin was observed in the main edible tissues (hepatopancreas, muscle, ovary and spermary), and the drug residue was the highest in the hepatopancreas, where the 'drug sink' phenomenon occurred. Comparative pharmacokinetics showed fast absorption, broad distribution and fast elimination of enrofloxacin in *E. sinensis* after intramuscular dosing. Regarding ciprofloxacin, the main active metabolite of enrofloxacin, though relatively low levels were detected in all the main tissues of the crab, its kinetics in the hemolymph in the two tests were not described by a one- or two-compartment open model.

Keywords: *Eriocheir sinensis*; Enrofloxacin; Ciprofloxacin; Pharmacokinetics

1. Introduction

Chinese mitten-handed crab (*Eriocheir sinensis*) is an economically important decapod crustacean in aquaculture in China. Although commercial production is rapidly expanding, the industry has been impeded by outbreaks of significant infectious diseases with serious economic consequences. Causative organisms of diseases in *E. sinensis* include viruses, bacteria, fungi and parasites. The bacteria that most commonly affect *E. sinensis* include *Aeromonas hydrophila*, *Spirillum* sp.,

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Flavobacterium sp., and numerous species of *Vibrio*, such as *V. alginolyticus*, *V. parahaemolyticus* and *V. anguillarum*. The use of antimicrobials to reduce such problems has become an important part of crab culture.

Due to its broad antibacterial spectrum and high potency, enrofloxacin, a member of the quinolone family, is commonly used to combat bacterial infection in crab farming in China. At present, the pharmacokinetics of enrofloxacin have previously been studied in poultry and mammals such as sheep (Haritova et al., 2003), dog (Helnen, 2002), goat (Rao et al., 2000), mare (Papich et al., 2002), rabbit (Cabanes et al., 1992), pig (Zeng and Feng, 1996), foal (Bermingham et al., 2000), and broiler chicken (Knoll et al., 1999). To our knowledge, studies of the pharmacokinetics and the main metabolite of enrofloxacin in aquatic animals have mainly focused on finfish species, including red pacu (*Colossoma brachypomum*) (Lewbart et al., 1997), rainbow trout (*Oncorhynchus mykiss*) (Bowser et al., 1992), seabream (*Sparus aurata*) (Rocca et al., 2004), seabass (*Dicentrarchus labrax*) (Intorre et al., 2000), and Atlantic salmon (*Salmo salar*) (Stoffregen et al., 1997). However, these studies were all done with teleosts, and there are no reports concerning the pharmacokinetics and the main metabolite of enrofloxacin in crustaceans.

The objective of the present study was to investigate the pharmacokinetics and main metabolite of enrofloxacin in Chinese mitten-handed crab (*E. sinensis*) after intramuscular administration. Such information will facilitate the appropriate use of enrofloxacin in reducing disease outbreaks in the aquaculture of Chinese mitten-handed crabs and other decapods.

2. Materials and methods

2.1. Experimental animals and chemicals

Healthy Chinese mitten-handed crabs (*E. sinensis*, mean weight 50.0±10.0 g) were obtained from a commercial farm in the suburbs of Shanghai, China. Six crabs taken before initiation of this study served as a negative control and were analyzed to confirm the absence of enrofloxacin and ciprofloxacin. The crabs were housed in fiberglass tanks (500 l) supplied with a constant flow (1 l/h) of aerated fresh water. The water quality was monitored daily, and adjusted as necessary. The pH was maintained between 6.4 and 7.0, while water temperature was adjusted to 25.0 ±1.0 °C by means of cooling and heating devices. The crabs were allowed to acclimatize for 2 weeks before antibiotic administration, and were fed a commercial crab diet (Fuzhou Seahorse Feed Co. Ltd., China) ad libitum daily, throughout the course of the experiment.

Enrofloxacin and ciprofloxacin hydrochloride were purchased from Sigma (St Louis, MO, USA). Unless otherwise indicated, the chemicals used were of analytical or high-performance liquid chromatography (HPLC) grade.

2.2. Intramuscular administration and sampling

Enrofloxacin solution for intramuscular administration was prepared by dissolving enrofloxacin in 0.1 M HCl prepared in 0.9% NaCl and adjusting the pH of the solution to 10 with 1 M NaOH. Two enrofloxacin concentrations (2.5 and 5.0 mg/ml) were used. Intramuscular doses of enrofloxacin were delivered through the arthrodial membrane at the junction of the fourth pereopod and the abdomen on the ventral side, into the muscle of the crab. Enrofloxacin doses were 10.0 or 20.0 mg/kg body weight, with the injection volume adjusted for every body weight of crab.

Crabs were sampled at 0.083, 0.25, 0.5, 1, 3, 6, 12, 24, 48, 96, 168 and 240 h post-dosing. Eight crabs (four females and four males) were sacrificed at each sampling. Hemolymph was sampled from the sinus cavity under the carapace with a 1-ml syringe wetted with ACD solution (Cai et al., 1983) as an anticoagulant. Hemolymph samples were instantly mixed with the same volume of ACD solution, and were kept frozen at −70 °C. Other tissues, including hepatopancreas, muscle (not from the injection site), ovary and spermary, were also collected at each sampling.

2.3. Sample preparation and extraction technique

Hemolymph samples were thawed and vortexed to assure homogeneity before extraction. Hemolymph samples (0.5 ml) were placed into 10-ml polypropylene centrifuge tubes and extraction solution (7.0 ml) added to each. The extraction solution consisted of 1 M HCl–acetonitrile (4/500, v/v). The tubes were vortexed for 2 min and centrifuged for 10 min at 8000 rpm and 4 °C. The resulting clear supernatant was dried using a vortex evaporator (Botongc Co. Ltd., Shanghai, China) and reconstituted in 1.0 ml of mobile phase. After filtration with 0.45-μm disposable syringe filter units equipped with cellulose acetate membranes (Shanghai Quandao, Co. Ltd., China), the filtrate was injected into the HPLC system.

Tissues (1.0 g of hepatopancreas, muscle, ovary and spermary blended in a meat mincer) were thawed and placed in 50-ml polypropylene centrifuge tubes, and 1 M NaCl solution (1.0 ml) and 0.2 M phosphate-buffered saline (pH 7.4) (1.0 ml) equal to the weight of the tissues were added. The tubes were vortexed for 2 min, and then