

Pilot Pharmacokinetic Study of Ketorolac in the blue crab, *Callinectes sapidus*

Souza A.R.¹; Lewbart G.A.^{1*}; Papich M.G.²

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1-Department of Clinical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA

2-Department of Molecular Biomedical Sciences, College of Veterinary Medicine, North Carolina State University, Raleigh, NC, USA

*Corresponding author's Email: greg_lewbart@ncsu.edu

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Introduction

Only recently has invertebrate pain begun to be recognized and described (Harrison, 1994; Hanke, 1997; Quesada, 2011; Magee, 2013; Sneddon, 2014). Some crustaceans respond to painful stimuli, such as the loss of an appendage, by a rubbing behavior of the affected area. Research has also shown there are opioid receptors within crustacean eye stalks (Harrison, 1994; Hanke, 1997; Elwood, 2009; Sneddon, 2014). In spite of these recent discoveries, there are still many questions remaining, such as if there is effective pain management for invertebrates (Rowley, 2005; Barr, 2011; Quesada, 2011). Decapod crustaceans like blue crabs (*Callinectes sapidus*) are an important commercial food species for human consumption and the highest value seafood product in North Carolina (An

Analysis of North Carolina's Seafood Industry: National and State Perspectives, 2013). Many crustacean species, including blue crabs, are also maintained as display animals and for research. Therefore, as part of any animal welfare program, effective pain management should be addressed.

The purpose of this study was to obtain preliminary information on the pharmacokinetics of the non-steroidal anti-inflammatory (NSAID) drug, ketorolac (Barr, 2011; Quesada, 2011; Magee, 2013). Ketorolac was chosen because it is a potent cyclo-oxygenase inhibitor (COX-inhibitor) that has high efficacy for treating pain in people (Cerreta, 2018). Blue crabs were chosen because they are easy to access in North Carolina and have been studied previously with regards to

pain management (Schock, 2010; Quesada, 2011; Zotti, 2016).

Materials and methods

Most of the crabs used in this study were purchased at a local seafood market; however, unfortunately, the health status of these animals was not optimal. Three of the animals were wild caught from Bogue Sound, NC, USA, and were housed in a recirculating unit at the Center for Marine Sciences and Technology (CMAST) in Morehead City, NC, USA. The seafood market crabs were housed in separate aquaria in Raleigh, NC, USA. Upon intake, all 9 crabs were weighed, sexed, and then randomly assigned to treatment groups of either 0.25mg/kg, 0.5 mg/kg, 0.75 mg/kg ketorolac. Time zero hemolymph was collected (at least 0.3 mL), which was obtained via cardiocentesis.

In order to deliver an accurate dose of ketorolac the drug was first diluted 1:10 with 0.9% sterile saline. Injections were given intracardiac (IC) and the crabs were monitored via Doppler (Parks Medical Electronics, Inc., Aloha, OR, USA). Hemolymph samples (0.3-0.4 mL) were collected from the heart or base of a hind limb at 1, 2, 4, 8, 12, and 24 hour time points. Those that were still living at the end of the study were euthanized with potassium chloride solution via an IC stick. Hemolymph were placed in cryovials and stored at -80 degrees prior to analysis.

Analysis

Ketorolac in hemolymph was analyzed with high-pressure liquid chromatography (HPLC) using a method that was previously for another study (Cerreta, 2018). The method underwent a partial validation using blank hemolymph pooled from untreated blue crabs fortified with ketorolac.

There were not sufficient time points and concentrations to perform pharmacokinetic analysis of these data.

Results

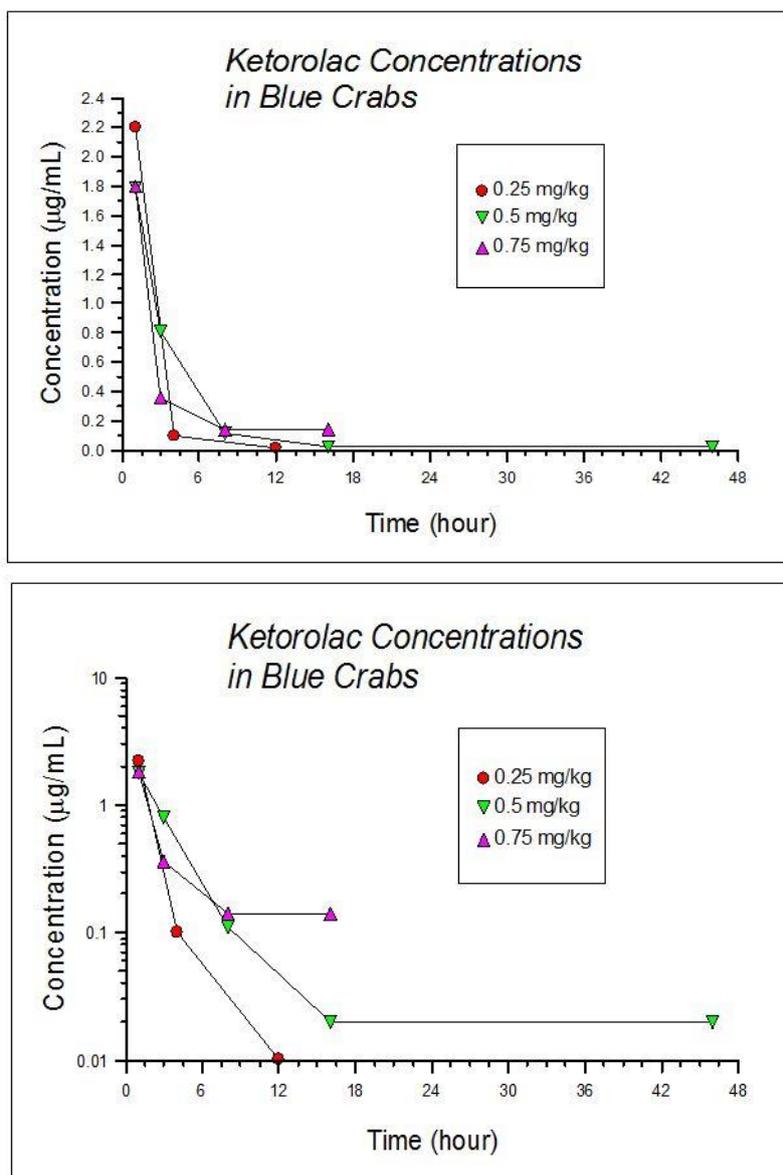
Concentrations of ketorolac measured after each dose is shown in Table 1 and Fig. 1. The data reported in this table and figure is just for the IM concentrations, IC concentrations are not reported due to the safety concerns of this route of administration.

A total of 9 blue crabs were treated with the average weight being around 127.9 grams. 3 crabs were treated with 0.25 mg/kg dose, 2 were treated with the 0.5 mg/kg dose, and 4 were treated with the 0.75 mg/kg dose. However, one 0.25 mg/kg dosed crab did not have any detectable ketorolac in the hemolymph samples drawn. The average lifespan of the IC blue crabs, regardless of the dose used, was around 2 hours; whereas, the crabs treated IM lasted an average of 28 hours, with one crab being humanely euthanized after sample collection.

Table 1: Summary of IM Ketorolac Concentration in Hemolymph Samples ($\mu\text{g/mL}$).

Time point (hours)	0.25 mg/kg	0.5 mg/kg	0.75 mg/kg
1	2.2	1.79	1.8
3	NS	0.81	0.36
4	0.10	NS	NS
8	NS	0.11	0.14
12	0.01	NS	NS
16	NS	0.02	0.14
24	ND	NS	
46		0.02	

NS: Not sampled; ND: Not detected

**Figure 1: Ketorolac concentrations in blue crabs. Linear axis on top, semi-logarithmic axis on bottom.**

Discussion

As expected, the 0.75 mg/kg IM dose produced higher values than the 0.25 mg/kg IM doses. Concentrations persisted longer with a higher dose. We showed that detectable concentrations of ketorolac can be achieved in these blue crabs, but the interpretation of the drug concentrations is not possible without additional study of what is considered “therapeutic” for these animals.

Our hypothesis was that the IC route would be most effective, yet, it was fatal to the blue crabs in our study. The IM ketorolac injection is preferred. Intramuscular ketorolac appeared more suitable and allowed long term-results to be obtained with more subjects surviving.

We hope to do more work utilizing fresh, wild caught blue crabs in the future. The uncertain history and husbandry of market crabs make them suboptimal for scientific study.

A longer pharmacokinetic study would be ideal with all crabs being wild caught and housed in separate flow through aquatic systems. The separate tanks would eliminate any possibility of drug contaminating other crabs, as well as disease or intraspecific trauma. The ketorolac dose used here that produced consistent concentrations was between 0.5 mg/kg and 0.75 mg/kg doses given intramuscularly (IM). The ultimate purpose would be to find an appropriate analgesic for crustaceans (in particular blue crabs), and find withdrawal times since these are a food animal.

Conclusions

The goal of this pilot study was to determine the pharmacokinetics of ketorolac in blue crabs using the IC and IM routes. Based on the results we determined IM is the preferred route and that ketorolac should not be given to unthrifty crabs.

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