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RESEARCH PAPER

The efficacy of alfaxalone for immersion anesthesia in koi carp (*Cyprinus carpio*)

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Abstract

Objective To characterize the physiologic and behavioral effects of a single induction dose and two maintenance doses of alfaxalone delivered by water immersion in the anesthesia of koi (*Cyprinus carpio*).

Study design Prospective, within-subject complete crossover design.

Animals Six adult koi (*Cyprinus carpio*) with a median body weight of 344.5 g (range 292.0–405.0 g).

Methods Koi were immersed in water containing 10 mg L^{-1} alfaxalone until immobile and then maintained with alfaxalone at either 1 or 2.5 mg L^{-1} via a recirculating water system. Times for anesthetic induction and recovery periods were recorded. Physiologic and blood gas parameters were evaluated before, during and after the anesthetic trial. Response to noxious stimuli was also assessed.

Results Median anesthesia induction time for all fish was 5.4 minutes. Median recovery time was 11.8 and 26.4 minutes in the 1.0 and 2.5 mg L^{-1} doses, respectively, which were significantly different ($p = 0.04$). Cessation of opercular movement occurred in 0/6 and 4/6 fish exposed to 1.0 and 2.5 mg L^{-1} dose respectively. No difference was observed in median heart rate over the duration of the anesthetic events. Response to noxious stimulation was 4/6 and 0/6 in the 1.0 and 2.5 mg L^{-1} doses respectively. Oxygenation and ventilation did not change during the experiment, but there was a significant decrease in blood pH along with an increase in blood lactate concentration.

Conclusion and clinical relevance Administration of alfaxalone, via water immersion, as an induction and maintenance anesthesia agent provided rapid and reliable anesthesia of koi with no mortality. The maintenance dose of 2.5 mg L^{-1} was sufficient to prevent response to noxious stimuli but was associated with a clinically relevant depression in opercular rate.

Keywords alfaxalone, Alfaxan, anesthetic, *Cyprinus carpio*, fish, koi carp.

Introduction

Cyprinid fish farming is a growing sector of the aquaculture industry in the United States, producing fish for ornamental purposes, food and bait (Goodwin 2002). Options for efficient, safe and predictable methods of anesthesia are needed to improve handling, transportation and manipulation of cyprinids. While the most commonly used and only FDA-approved fish anesthetic is tricaine methanesulfonate

(MS-222), a multitude of different anesthetic agents, including clove oil (both eugenol and isoeugenol), metomidate, and benzocaine, are currently being used by facilities which handle, manipulate and transport fish (Soivio et al. 1977; Smit et al. 1979a, b; King et al. 2005; Carter et al. 2011; Di Marco et al. 2011).

Alfaxalone, a progesterone analog, is a synthetic neuroactive steroid, which produces anesthesia and muscle relaxation by selective modulation of the gamma aminobutyric (GABA) receptor, which has been identified in various areas of the brain of teleost fish (Cottrell et al. 1987; Doldan et al. 1999). It was previously marketed for veterinary use under the name Saffan (Schering Plough Animal Health, NJ, USA) in a co-formulation with alfadolone, another related steroid, but was discontinued because the solubilizing agent, Cremophor EL, was associated with histamine release in both dogs and cats (Sear 1996). Saffan was used successfully for both mechanoreceptor and chemoreception research in perch (*Perca fluviatilis*) and African catfish (*Clarias gariepinus*) (Peters et al. 2001). A new formulation of alfaxalone without alfadalone (Alfaxan; Jurox Pty. Ltd, Australia) has been solubilized with 2-hydroxypropyl beta-cyclodextrin and is currently registered in several countries for intravenous and intramuscular induction and maintenance of anesthesia in dogs and cats (Maddern et al. 2010; Taboada & Murison 2010). Recently, there have been several citations in the literature describing induction and maintenance of anesthesia in both fish and amphibians with alfaxalone delivered via a water bath (O'Hagan & Raidal 2006; Bauquier et al. 2011; McMillan & Leece 2011).

The objective of this study was to evaluate the efficacy of a single induction dose and two maintenance doses of alfaxalone delivered by water immersion in the anesthesia of koi (*Cyprinus carpio*) by evaluating dose related changes in serial blood gas values, blood lactate concentrations and behavioral effects. We hypothesized that the induction dose (10 mg L^{-1}) and both maintenance doses (1 and 2.5 mg L^{-1}) would be sufficient both to induce and maintain anesthesia in koi.

Materials and methods

Animals

Six adult koi (median weight: 344.5 g, range: 292.0–405.0 g) were group housed in an indoor system comprised of a 1500-L fiberglass circular tank filled with dechlorinated municipal water maintained at room temperature. A photoperiod of 12-light:12-dark hours was maintained. Fish were allowed to acclimate to this system for 8 weeks prior to the experiment. Water temperature (range 17.4–18.5 °C), pH (range 6.9–7.6), total ammonia concentration (range 0.0–0.25 mg L^{-1}) and nitrate (range 0.0–5.0 mg L^{-1}) were monitored and recorded on a daily basis. A 50% water change was performed every 2 weeks. Fish were observed daily and fed a pelleted diet formulated for ornamental koi (Blackwater farms Maximum growth; Blackwater Creek Koi Farms Inc., FL, USA) three times per week at 1% body weight for maintenance. All fish were considered healthy following a visual examination and external parasite evaluation, performed using light microscopy on fresh mounts of both skin scrapes and gill biopsies. All procedures were reviewed and approved by the Institutional Animal Care and Use Committee.

Study design and procedure

Each fish was exposed to a single induction dose (10 mg L^{-1}) and two maintenance doses (1.0 and 2.5 mg L^{-1}) of alfaxalone in a randomized within-subject complete crossover design, allowing for a washout period of 3 weeks between each of the anesthetic exposures. Doses of alfaxalone were chosen based on pilot data from this laboratory. Before exposure to alfaxalone, fish were removed from the holding tank and using a 25-gauge needle attached to a 1 mL syringe, a 0.15 mL blood sample was obtained via the caudal artery or vein. Blood CO_2 and O_2 partial pressure (PCO_2, PO_2), pH, bicarbonate concentration (HCO_3) and lactate concentrations were determined using an iStat Portable Clinical Analyzer with CG4+ cartridges (Heska Corp, NJ, USA). Blood CO_2 and O_2 partial pressure, pH and lactate concentrations are measured directly by the portable clinical analyzer. Bicarbonate ion concentration was calculated from directly measured values by the portable clinical analyzer utilizing the Henderson–Hasselbalch equation. All blood values were reported at an analyzer temperature of 37 °C, recognizing that a variety of temperature corrections could be employed other than those applied by the iStat portable clinical analyzer.

After the initial blood collection, fish were placed into a prefilled static immersion bucket containing 8 L of water, obtained from the same system from

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