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RESEARCH PAPER

Physiologic and biochemical measurements and response to noxious stimulation at various concentrations of MS-222 in Koi (*Cyprinus carpio*)

Jonathan Stockman*, Ernest Scott P Weber III*†, Philip H Kass‡, Peter J Pascoe§ & Joanne Paul-Murphy¶

*William R. Pritchard Veterinary Medical Teaching Hospital (VMTH), School of Veterinary Medicine, University of California, Davis, CA, USA

†Center for Aquatic Biology & Aquaculture (CABA), University of California, Davis, CA, USA

‡Department of Population Medicine and Immunology, School of Veterinary Medicine, University of California, Davis, CA, USA

§Department of Surgical and Radiological Sciences, School of Veterinary Medicine, University of California, Davis, CA, USA

¶Department of Medicine and Epidemiology, School of Veterinary Medicine, University of California, Davis, CA, USA

Correspondance: Joanne Paul-Murphy, Department of Veterinary Medicine and Epidemiology, School of Veterinary Medicine, University of California, One Shields Ave, Davis, CA 95616, USA. E-mail: paulmurphy@ucdavis.edu

Abstract

Objective To evaluate the physiological effect and response to noxious stimulation at five concentrations of MS-222 in koi (*Cyprinus carpio*).

Study design Prospective experimental study.

Animals Twenty-one healthy adult unknown sex koi fish weighing mean 450 ± SD 120 g.

Methods Each fish was exposed to five different concentrations of MS-222 (50, 70, 110, 150 and 190 mg L^{-1}) in a random sequence during the same anaesthetic event. For each concentration of MS-222, vital functions such as heart rate (HR) (via Doppler) and opercular rate (OpR) were recorded after a standardized induction period. Response to two noxious stimuli in the form of haemostat clamp pressure applied on the tail and the lip was evaluated, and blood was drawn to measure biochemical and blood gas values.

Results Decrease in response to noxious stimulation with an increase of MS-222 concentration both for the lip ($p = 0.0027$) and the tail ($p < 0.0001$) stimulus was observed. Biochemical values were unaffected by the concentration of MS-222 with the exception of lactate concentration which was weakly correlated with the duration of anaesthesia ($r = 0.31$, $p < 0.001$) and the number of times the fish was clamped or bled prior to sampling ($r = 0.23$, $p < 0.001$). Opercular rate decreased with the increase in anaesthetic concentration, and HR was not affected.

Conclusions and clinical relevance Our results indicated a decrease in response to stimulus and a decrease in OpR that were associated with increased concentrations of MS-222. This may assist in establishing anaesthetic protocols using MS-222 in fish and supports the use of supramaximal pressure stimuli to teleost fish under variable MS-222 concentrations as a model for future studies.

Keywords anaesthesia, blood gas, Koi, MS-222, noxious stimuli, supramaximal stimulus.

Introduction

Veterinary expertise in fish health has increased in aquaculture, laboratory animal medicine, zoos and public aquariums, wild fisheries, and private practice with ornamental fish over the last two decades (Weber et al. 2009a,b). Immobilization is required for most procedures from phlebotomy to surgery, due to the difficulty of fish restraint, handling associated stressors, and the possibility of trauma. Different anaesthetic agents are available, and although injectable substances are used regularly for many vertebrate patients, fish are most often anaesthetized using a bath containing water-soluble anaesthetics. Agents commonly used include metomidate hydrochloride, quinaldine and quinaldine sulfate, benzocaine, carbon dioxide (CO_2), isoeugenol, eugenol (clove oil) and tricaine methanesulfonate (MS-222; Western Chemical, WA). MS-222 is the most widely used substance for fish anaesthesia and is currently the only federally approved fish anaesthetic in the United States, although the US Food and Drug Administration under the Minor Use and Minor Species (MUMS) health act of 2001 has recently indexed metomidate hydrochloride for sedation and anaesthesia of ornamental finfish.

MS-222 is readily soluble in water and is also lipid soluble, enabling it to be transported rapidly across the gills (Pirhonen & Schreck 2003). The addition of MS-222 to water commonly is given within a recommended range of 15–330 mg L^{-1} (Bowser 2001), but generally is subjectively added to effect. The compound is excreted readily across the gills during recovery and the tissue concentration decreases to near zero within 24 hours after recovery (Bowser 2001) depending upon the concentration of MS-222 used and exposure time. It has been shown in several fish species that MS-222 metabolism is hepatic (Wayson et al. 1976) and utilizes cytochrome P-450 enzymes. However the metabolism rate and induction of cytochrome P-450 differs between fish species (Fabacher 1982), therefore the effective dose could be variable. Despite its use in fish for invasive procedures such as coelomic surgery (Weisse et al. 2002), the analgesic effect of MS-222 has not been scientifically documented.

Fish possess nociceptors and their nature and distribution have been described and documented on the head and lip area of several species such as the sea lamprey (Matthews & Wickelgren 1978) and the rainbow trout (*Oncorhynchus mykiss*) (Sneddon et al. 2003). Noxious stimulation is defined as that which is produced by electrical, chemical, thermal or mechanical stimulation. The sensation of noxious stimulation in several fish species has been documented both *in vivo* and *in vitro* (Matthews & Wickelgren 1978; Sneddon 2003a,b; Sneddon et al. 2003; Ashley et al. 2007). Several of these studies have used noxious stimuli (pinching, puncturing, and burning) applied to the skin and documented the electrophysiological activity of trigeminal ganglia innervating the fish's head (Matthews & Wickelgren 1978; Sneddon 2003a,b; Ashley et al. 2007; Newby 2008; Nordgreen et al. 2009). Anatomical and physiological studies have confirmed the existence of both A-delta and C nociceptor fibers in rainbow trout (Sneddon 2002, 2003a,b). As in tetrapods, these fibers in the trout trigeminal nerve have polymodal, slow adapting receptors activated by mechanical, thermal and chemical stimuli (Sneddon 2002, 2003a,b).

Pain and distress are undesired outcomes for any animal while undergoing diagnostic testing, being handled, transported, or subjected to medical treatments and/or surgery, because they may lead to cortisol release and subsequent immunosuppression (Thomas & Robertson 1991). Several immune modulator genes have been shown to change expression in response to increased cortisol concentration and to stress in fish (Castillo et al. 2009). Cardiovascular effects, such as changes in cardiac output and arterial blood pressure, have also been shown to occur as a result of noxious stimulation such as surgery during an anaesthetic procedure in fish (Rothwell et al. 2005; Newby 2008). Complex behavioural patterns have been documented in fish in response to nociceptive stimulation in addition to simple withdrawal responses (Chervova & Lapshin 2000; Sneddon et al. 2003; Nordgreen et al. 2009). These behaviours include 'rocking' on the bottom of the tank and rubbing of the mouth in response to locally injected noxious agents (Sneddon 2003a,b).

Changes in blood parameters of teleost fish undergoing anaesthesia using MS-222 have been documented (Soivio et al. 1977; Smit et al. 1979; Harms et al. 2005) and previous studies have evaluated the response to needle placement in the epaxial muscles or to phlebotomy in red pacu fish (*Piaractus brachypomus*) and anaesthetized Chain dogfish (*Scyliorhinus retifer*) under serial MS-222 or clove oil concentrations (Sladky et al. 2001; Davis et al. 2006). However, it is unknown how various concentrations of MS-222 affect fish physiology and response to supramaximal noxious stimuli. A supramaximal noxious stimulus is defined as the minimal stimulation beyond which there is no