Immersion anesthesia in goldfish (Carassius Auratus) with three different concentrations of alfaxalone.

ABSTRACT

Objective To evaluate the anesthetic effects and the reliability of three different alfaxalone doses not previously used to induce anesthesia in goldfish.

Study design Prospective, randomized, clinical study.

Animals Thirty goldfish undergoing skin scraping, gill exam and stool collection.

Methods Each fish was transferred to an individual 4 L induction tank. The fish were randomly allocated into three groups (n = 10) in which anesthesia was induced with alfaxalone 6 mg/L, 7 mg/L and 9 mg/L. The depth of anesthesia was evaluated by assessing reactivity, activity, maintenance of equilibrium, opercular movement and response to noxious stimuli. Sedation, light anesthesia and surgical anesthesia induction times and recovery time were recorded. The fish length (from snout to fork in caudal fin) was measured. The data were analyzed using ANOVA. Statistical significance was set at $p < 0.05$

Results All fish achieved surgical anesthesia stage. Goldfish induced with alfaxalone 7 mg/L and 9 mg/L showed a mild excitement phase. Sedation induction time of 6 mg/L dose was significantly longer compared to 7 mg/L and 9 mg/L doses. Light anesthesia and surgical anesthesia induction times of 9 mg/L dose were significantly faster compared to 6 mg/L and 7 mg/L doses. No significant differences were recorded in recovery time. Induction and recovery times had no correlation with goldfish size. The cessation of opercular movement was recorded in two fish induced with 7 mg/L and in two induced with 9 mg/L. At 15 days post anesthesia, the fish had a normal physical appearance and no death was observed.

Conclusions and clinical relevance Alfaxalone is a reliable agent for immersion anesthesia in goldfish. Immersion in water concentration of 6 mg alfaxalone/L provides a smooth induction of anesthesia without side effects. Higher doses shorten the time required for induction, and cause respiratory depression and excitatory movements.

Keywords goldfish, Carassius Auratus, fish, immersion anesthesia, alfaxalone.

INTRODUCTION

Veterinarians always more often treat ichthyic sector. Goldfish (*Carassius auratus*) is the most commonly fish kept as pet. Goldfish is a long-lived species and it may be affected by cutaneous, intestinal, metabolic disorders and neoplasm (O'Hagan & Raidal 2006). Sedation and general anesthesia are required to handling, diagnostic tests and surgical procedures (Sneddon 2012).

The key points of fish anesthesia are how to assess the depth of anesthesia, which route to use for administering drugs and what drug to employ. The depth of anesthesia may be evaluated assessing reaction to external stimuli, swimming, gill ventilation rate and reflex response (Sneddon 2012). Furthermore, the depth of anesthesia is related to the anesthetic agent, dose and time exposure (Fleming et al. 2003; West et al. 2007).

Immersion anesthesia is more often performed than parenteral anesthesia (West et al. 2007). Sedative and anesthetic agents used for fish are very different from those used for mammalians. The most common drugs used to induce general anesthesia in fish are as follow: tricaine (MS-222), benzocaine, isoeugenol and quinaldine (Sneddon 2012). Many agents, which are usually employed for parenteral anesthesia in dogs and cats, are water-soluble and may be administered through the water because fish ventilate the anesthetic agent in solution (Sneddon 2012). Medetomidine-ketamine (Fleming et al. 2003), atipamezole (Williams et al. 2004), propofol (Fleming et al. 2003; GholipourKanani & Ahadizadeh 2013), metomidate (Iversen et al. 2003), and diazepam (Kumlu & Yanar 1999) produced variable results in fish.

A formulation composed of a mixture of alfaxalone and alphadolone acetate solubilized in 20% polyethoxylated castor oil (Cremophor-EL) has been previously employed as an intramuscular or an immersion anesthetic agent in fish (Harvey et al. 1988; Peters et al. 2001). A new water-soluble formulation of alfaxalone solubilized in 2-hydroxypropyl-beta cyclodextrin (Ferre et al. 2006) has been employed as an immersion anesthetic agent. In koi carp (*Cyprinus carpio*), immersion in water concentration of 10 mg alfaxalone/L caused fast induction, but the cessation of opercular movement was detected during maintenance (Minter et al. 2014). In oscar fish (*Astronotus ocellatus*), immersion anesthesia with alfaxalone 5 mg/L was reliable for collection of blood samples, despite it significantly reduced the respiratory rate (Bugman et al. 2016). The use of alfaxalone in goldfish was described in two case reports (O'Hagan & Raidal 2006; Fernández-Parra et al. 2017). Induction and maintenance were achieved with alfaxalone, respectively, 10 mg/L and 5 mg/L, but respiratory depression occurred (Fernández-Parra et al. 2017). Moreover, the findings of Bauquier et al. (2013) underscored that administering alfaxalone 5 mg/L induced surgical anesthesia in 5/6 goldfish and light anesthesia in 1/6 goldfish whereas 7.5 mg/L dose induced surgical anesthesia in 6/6 goldfish but caused delayed recovery compared to 5 mg/L dose (Bauquier et al. 2013).

The purpose of this study is to evaluate the reliability of three different alfaxalone doses not previously used to induce general anesthesia in goldfish. The present clinical study aims to assess the anesthetic effects of 6 mg, 7 mg and 9 mg alfaxalone/L and to compare the effect of these doses on induction and recovery times.

MATERIALS AND METHODS

Animals

The study was performed in accordance with the XXX legislation on animal care (XXX) and XXX law (XXX).

Thirty goldfish of unknown gender, one year-old, obtained from the same fish private pond, that underwent skin scraping, gill exam and stool collection, were enrolled. For the acclimation period of 15 days, thirty goldfish were housed indoors together in 130 L tank filled with municipal water constantly aerated with an air stone on a mechanical pump (Haquoss airline3 180 L/h, Haquoss, XXX). Water temperature, pH and nitrates were daily measured and ranged between, respectively, 22.4-24°C, 6.9-7.5 and 0.1-0.23 mg/L. The fish were fed with balanced dry fish food given twice a day. They were considered healthy following a visual exam based on the evaluation of equilibrium, swimming, opercular movement and physical appearance.

Study design

The fish were withheld for 12 hours before anesthesia. Each fish was transferred to an individual 4 L induction tank filled to 75% and aerated with an air stone on a mechanical pump. The water used for trials was the same in which the fish were housed. Two g/L of NaCl was added to each induction tank to help fish osmoregulation.

The fish were randomly (simple randomization) divided into three groups (n = 10) in which anesthesia was induced, using three different concentrations of alfaxalone (Alfaxan, Dechra, XXX) as follow: 6 mg/L (group G6), 7 mg/L (group G7) and 9 mg/L (group G9).

Before adding alfaxalone to the anesthetic tank, the fish was observed for approximatively 10 minutes and the approach reaction score was evaluated (Table 1). After adding alfaxalone to the induction tank, the assessment of approach reaction, equilibrium and operculum movement scores (Table 1) was performed every minute until loss of equilibrium and reaction to tactile stimulus. The anesthetic stages were evaluated according to criteria outlined in Table 2. When fish reached the anesthetic stage 3, response to noxious stimuli was assessed by the same practitioner that squeezed the caudal fin between two fingers. When response to noxious stimuli score reached 4 (Table 1), the fish was removed from the anesthetic bath, laid down on a gauze dampened with water and photographed. The fish length (from snout to fork in caudal fin) was measured and skin scraping, gill exam and stool collection were performed in two minutes. At the end of these procedures, the fish was transferred to an individual 4 L recovery tank filled to 75% and aerated with an air stone on a mechanical pump. The fish was gently moved with a steel stick in a swimming motion until righting reflex resumed. If opercular movement was not detected, a steady flow of water was directed through the oral cavity and across the gills. The fish was moved to the original 130 L tank after recovery.

A daily visual exam based on the evaluation of equilibrium, swimming, opercular movement and physical appearance was performed for 15 days.

Data collection

The recorded times (minutes) were defined as follow: sedation induction time (the time from adding alfaxalone to water to the time of sedation stage), light anesthesia induction time (the time from adding alfaxalone to water to the time of light anesthesia stage), surgical anesthesia induction time (the time from adding alfaxalone to water to the time of surgical anesthesia stage), sedation – light anesthesia (the time from sedation stage to light anesthesia stage), light anesthesia – surgical anesthesia (the time from light anesthesia stage to surgical anesthesia stage), sedation – surgical anesthesia (the time from sedation stage to surgical anesthesia stage) and recovery time (the time from the fish transfer to the recovery tank to the time of recovery of normal approach reaction, equilibrium and operculum movement scores).

Statistical analysis

Fish length was compared using Students *t* test. Fish length was reported as mean ± standard deviation (SD).

ANOVA analysis was performed to evaluate the times using the general linear model (GLM) procedure with software package IBM SPPSS Statistics vers. 20 (IBM Corp., Armonk, NY, USA) with the alfaxalone concentration (three levels: 6 mg/L, 7 mg/L and 9 mg/L) as fixed factor. Later, the times were covariated with the fish length. The times in minutes (min) were reported as least-squares means (LSM) ± standard error of the mean (SEM). P values < 0.05 were considered significant.

RESULTS

Fish length

Mean fish length was 78.1 ± 15.4 mm. Mean fish lengths in the three groups were as follow: 81.8 ± 6.8 mm (group G6), 72.2 ± 23.6 mm (group G7) and 80.2 ± 12.5 mm (group G9). There were no significant differences between groups.

Anesthetic effects

After adding alfaxalone to the induction tank, the fish belonged to the groups G7 and G9 showed a short excitement period during which they rapidly swam forward and backward across the tank or upright with its mouth facing the surface of the water or the bottom of the tank. This behavior approximatively lasted two minutes.

Two fish belonged to the group G7 showed the cessation of opercular movement when surgical anesthesia stage was achieved. The fish were immediately removed from the anesthetic bath and a steady flow of water was directed through the oral cavity and across the gills. Two fish belonged to the group G9 required the same treatment because of the cessation of opercular movement when they were transferred to the recovery tank. All four fish recovered slow regular opercular movement in one minute after the administration of the steady flow of water across their gills.

Induction and recovery times

All the goldfish achieved surgical anesthesia stage.

Sedation, light anesthesia, surgical anesthesia induction times and recovery time are reported in Table 3. In the group G6, sedation induction (6.00 min) time was significantly longer compared to those of the groups G7 (3.80 min) and G9 (4.00 min). Light anesthesia and surgical anesthesia induction times in the group G9 (8.00 and 10.20 min) were significantly faster compared to those of the groups G6 (14.40 and 20.80 min) and G7 (12.60 and 19.60 min). No significant difference was recorded in recovery time between groups.

The change in anesthetic depth was significantly faster in the group G9 compared to those of the groups G6 and G7 (Table 4).

Fish length did not influence statistical significances (Tables 5 and 6).

Follow up

At 15 days post anesthesia, all the fish had normal equilibrium, swimming, opercular movement and physical appearance, and no death was recorded.

DISCUSSION

This study demonstrates that alfaxalone 6 mg/L, 7 mg/L and 9 mg/L doses induce surgical anesthesia stage in goldfish. Immersion in water concentration of 9 mg alfaxalone/L provides a faster induction of light and surgical anesthesia stages compared to 6 mg/L and 7 mg/L doses. The cessation of opercular movement may occur with immersion in water concentration of 7 mg and 9 mg alfaxalone/L.

Many water-soluble agents have been used for immersion anesthesia in fish (Harms 1999). The ideal anesthetic agent must provide a reliable induction and adequate recovery and it should be routinely stocked by the practitioners in their clinics. Propofol and alfaxalone were used to induce and maintain general anesthesia in goldfish (Fleming et al.; 2003; Bauquier et al. 2013; GholipourKanani & Ahadizadeh 2013; Fernández-Parra et al. 2017).

Alfaxalone is a neuroactive steroid that induces sedation and anesthesia because it selectively modulates gamma aminobutyric receptors (Cottrell et al. 1987). These receptors have also been identified in the brain of fish (Cottrell et al. 1987). Nevertheless, there are doubtful results as regard alfaxalone concentration to induce surgical anesthesia in goldfish. Bauquier et al. (2013) showed that alfaxalone concentration of 5 mg/L was satisfactory to induce surgical anesthesia in 83% of the sample and 7.5 mg/L dose produced surgical anesthesia in 100% of the sample. In other findings, alfaxalone 5 mg/L induced surgical anesthesia and the fish were able to maintain neutral buoyancy at 15-35 minutes after the end of anesthesia (O'Hagan & Raidal 2006; Fernández-Parra et al. 2017). Our data underscore that fish anesthetized with alfaxalone 6 mg/L, 7 mg/L and 9 mg/L achieved surgical anesthesia. Potential causes of these differences include physical-chemical characteristics of the water, and biological factors such as age, size, body condition and ratio of gill area to body mass.

Water temperature, pH and osmolality influence metabolism in fish and therefore the values of these parameters are important to understand the results. Lower water temperature is associated with prolonged anesthetic induction and delayed recovery (Neiffer & Stamper 2009). Given that goldfish are housed in cold water, it is reasonable to presume that induction times are prolonged and recovery times are delayed compared to those of fish housed in warm water. Lower water pH increases ionization and decreases anesthetic agent efficacy (Neiffer & Stamper 2009). The addition of salt to the anesthetic bath is recommended because anesthesia alters fish osmoregulation (Sneddon 2012). Furthermore, the sudden change in water temperature, pH and osmolality causes stress which results in an increased gill blood flow producing a greater anesthetic absorption (West et al. 2007). To reduce the influence of these factors on immersion anesthesia and to minimize the stress for the fish, we used the water obtained from the house tank for the anesthetic bath and recovery.

In this study, induction and recovery times had no correlation with goldfish size. In the veterinary literature, there are no previous findings focused on relationship between goldfish size and anesthetic requirements. In Atlantic salmon and brown trout, size did not influence induction and recovery times (Sneddon 2012). Conversely, larger body size in whitefish, Senegalese sole and Atlantic cod was associated with increased or decreased induction and recovery times (Sneddon 2012). The likely reason for these differences could be that each anesthetic agent has a specie-specific effect because of lipid solubility and lipid content of fish, basal metabolism and gill surface area (Gressler et al. 2012; Sneddon 2012).

The induction times recorded in this study were those to be expected based on the available literature (O'Hagan & Raidal 2006; Bauquier et al. 2013; Fernández-Parra et al. 2017). Moreover, as expected, 9 mg/L dose showed faster induction times compared to 6 mg/L and 7 mg/L doses. The explanation of these results can be that high doses of alfaxalone may cause hypotension and reflex tachycardia, which lead to an increase in blood flow though the gills and, consequently, an increase in alfaxalone intake.

Our results underscored that alfaxalone dose had no significant influence on recovery time and contradicted an expectation that higher alfaxalone concentration could increase recovery time. In fact, in koi carp a higher alfaxalone dose (2.5 mg/L) produced delayed recovery compared to 1 mg/L dose (Minter et al. 2014). Additionally, although an ideal recovery from general anesthesia must be lower than 10 minutes, the recovery times recorded in our sample were satisfactory and lower compared to those reported in goldfish (O'Hagan & Raidal 2006; Bauquier et al. 2013; Fernández-Parra et al. 2017) and koi carp (Minter et al. 2014). The likely reason for the observed differences is that surgical anesthesia was maintained for two minutes in the present study, a time significantly shorter compared to other reports (O'Hagan & Raidal 2006; Minter et al. 2014; Fernández-Parra et al. 2017).

Higher alfaxalone doses caused excitatory movements. Side effects previously described in fish anesthetized with alfaxalone are as follow: unilateral horizontal nystagmus in koi carp (Minter et al. 2014) and increased hearth rate in oscar fish (Bugman et al. 2016). We did not record any of these side effects, but an interesting observation is that a hyperactivity period was recorded after adding alfaxalone to the induction thank. In goldfish, the authors are unaware of previous findings reporting a hyperactivity period after adding alfaxalone. However, a short excitement period was described during induction phase of immersion anesthesia in some fish species (Stetter 2001). The mild excitement phase may be due to the chemical irritation of the gills.

Respiratory depression is the most common side effect recorded during immersion anesthesia. In the present study, cessations of opercular movement were recorded with high alfaxalone doses, similarly to a previous finding (Fernández-Parra et al. 2017). One explanation of these results is that higher doses speed up the change in the depth of anesthesia and, if anesthetic induction is too fast, differentiating one anesthetic stage from another may be difficult and side effects may rapidly arise.

The cessation of opercular movement can lead to hypoxia that induces gills filament collapse and delayed recovery (Harms 1999). Notwithstanding the cessation of opercular movement can be resolved by transferring the fish to a recovery tank (Fernández-Parra et al. 2017), we preferred to deliver a steady flow of oxygenated water through the oral cavity and across the gills in order to stimulate the buccal flow/heart rate reflex. Although goldfish are anoxia tolerant compared to other species (Bickler & Buck 2007), a constant monitoring of anesthetic depth is required to avoid cardiorespiratory complications.

A limitation of this study is the assessment of depth of anesthesia. Activity, equilibrium, reaction to tactile or noxious stimuli and opercular movement have been widely used to determine the depth of anesthesia in fish, but these parameters depend on species, drug and dose (Neiffer & Stamper 2009; Sneddon 2012; Bauquier et al. 2013). In the present study, the response to noxious stimulus was assessed with a pressure applied to the caudal dorsal fin. The disadvantage of this method was the inaccurate repeatability of applied force.

In conclusion, the present study shows that alfaxalone may be considered a reliable anesthetic induction agent in goldfish. Immersion in water concentration of 6 mg alfaxalone/L provides a smooth induction of surgical anesthesia stage in approximatively 20 minutes without side effects. Higher doses shorten induction times, and cause respiratory depression and excitatory movements. Induction and recovery times have no correlation with goldfish size. Additional studies are needed to evaluate the efficacy and safety of alfaxalone for a longer duration.

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TABLES

Table 1. Assessment of approach reaction, equilibrium, operculum movement and reaction to noxious stimuli using ordinal scales (Bauquier et al. 2013, modified)

Approach reaction score		
	0	Normal: swims away when approached
	1	Reduced: slower to react and slower swimming
	2	Low: slow to react and may not react reliably
	3	Lost: no reaction to approach or contact
Equilibrium score		
	0	Normal: strongly retains upright position when still and swimming
	1	Reduced: wobbles from upright position when still and swimming
	2	Low: leans or lies on side or may turn upside down, returns to upright when swimming or when stimulated
	3	Lost: leans or lies on side or may turn upside down, does not return to upright and generally does not swim
Operculum movement score		
	0	Normal: rate will be recorded
	1	Reduced: slowing of the operculum rate
	2	Slow: slow but steady rate
	3	Lost: no operculum movement
Response to noxious stimuli score		
	0	Normal: strong tail wiggle (at least 5 movements in 1 to 2 s)
	1	Reduced: reduced tail wiggle (3 to 5 movements in 1 to 2 s)
	2	Slow: weak tail wiggles (1 to 3 movements in 1 to 2 s)
	3	Faint: tail flinch only (1 movement in 1 to 2 s)
	4	Lost: no tail wiggles or movement

s, seconds.

Table 2. Anesthetic stages of fish (Tranquilli et al. 2007, modified)

Stages	**Level of consciousness**	**Behavior**
Stage 0	Normal	Normal equilibrium, normal operculum movement, normal response to visual and tactile stimuli
Stage 1	Sedation	Normal to reduced equilibrium, reduced operculum movement, reduced response to visual and tactile stimuli
Stage 2	Light anesthesia	Loss of equilibrium, reduced to slow operculum movement, loss reaction to visual stimuli, slow to faint response to tactile stimuli
Stage 3	Surgical anesthesia	Loss of equilibrium, slow operculum movement, loss of reaction to visual or tactile stimuli
Stage 4	Medullary collapse	Loss of equilibrium, loss of operculum movement, loss of reaction to visual and tactile stimuli, cardiac arrest and death

Table 3. Sedation, light anesthesia, surgical anesthesia induction times and recovery time in minutes (expressed as LSM ± SEM) in the three groups

Anesthetic stages	**Group**				
	G6	**G7**	**G9**	SEM	*p*
Sedation	6.00[b]	3.80[a]	4.00[a]	0.400	0.031
Light anesthesia	14.40[b]	12.60[b]	8.00[a]	1.040	0.020
Surgical anesthesia	20.80[b]	19.60[b]	10.20[a]	1.843	0.022
Recovery	14.80	18.20	16.80	1.077	0.465

[a,b]Significant differences in the row between groups. The alphabetical order indicates the order of the data.

Table 4. Intervals of time in minutes (expressed as LSM ± SEM) elapsed between sedation and light anesthesia, light anesthesia and surgical anesthesia, sedation and surgical anesthesia

Intervals of time	**Group**				
	G6	**G7**	**G9**	SEM	*p*
Sedation - light anesthesia	8.40[b]	8.80[b]	4.00[a]	0.896	0.038
Light anesthesia - surgical anesthesia	6.40[b]	7.00[b]	2.20[a]	0.981	0.084
Sedation - surgical anesthesia	14.80[b]	15.80[b]	6.20[a]	1.741	0.032

[a,b]Significant differences in the row between groups. The alphabetical order indicates the order of the data**.**

Table 5. Data covariated with the fish length. Sedation, light anesthesia, surgical anesthesia induction times and recovery time in minutes (expressed as LSM ± SEM) in the three groups

Anesthetic stages	**Group**				
	G6	**G7**	**G9**	SEM	*p*
Sedation	5.89[b]	3.97[a]	3.94[a]	0.400	0.046
Light anesthesia	13.79[b]	13.55[b]	7.65[a]	1.040	0.000
Surgical anesthesia	19.75[b]	21.24[b]	9.60[a]	1.843	0.001
Recovery	14.40	18.82	16.57	1.077	0.288

[a,b]Significant differences in the row between groups. The alphabetical order indicates the order of the data.

Table 6. Data covariated with the fish length. Intervals of time in minutes (expressed as LSM ± SEM) elapsed between sedation and light anesthesia, light anesthesia and surgical anesthesia, sedation and surgical anesthesia

Intervals of time	**Group**				
	G6	**G7**	**G9**	SEM	*p*
Sedation - light anesthesia	7.90[b]	9.58[b]	3.71[a]	0.896	0.002
Light anesthesia - surgical anesthesia	5.96[b]	7.69[b]	1.95[a]	0.981	0.028
Sedation - surgical anesthesia	13.86[b]	17.27[b]	5.66[a]	1.741	0.002

[a,b]Significant differences in the row between groups. The alphabetical order indicates the order of the data.