IRANIAN JOURNAL OF
VETERINARY SURGERY
(IJVS)
WWW.IVSA.IR

Anesthesia with Propofol in Grass Carp, *Ctenopharyngodon idella,* and its Effects on Electrocardiogram, Blood Gases and pH

Rahim Peyghan [*1], PhD
Ahmad Ali Papahn [1], PhD
Hadi Nadaf [1], PhD
Ahmad Ebadi [2], PhD

[1]Department of Clinical Sciences, Faculty of Veterinary Medicine, Shahid Chamran University, Ahvaz, Iran,
[2]Ahvaz Jondishapour University of Medical Sciences, Ahvaz, Iran.

Abstract

Objective- This study aims to determine the efficacy of propofol in grass carp anesthesia and to examine the impact of the drug on electrocardiogram, pH and blood gases in this fish species.
Design- Experimental study.
Animals- 120 apparently healthy grass carps, weighting between 1-2 kg.
Procedure- Fishes were sorted randomly into 12 groups of 10 fish each. Five groups were anesthetized by bath method with the concentrations of 2, 4, 6, 8 and 10 mg/l and the other 5 groups by injection method with doses 2, 4, 6, 8 and 10 mg/kg, IV. Two groups were considered as control. At time of anesthesia and recovery, arterial blood samples from dorsal aorta of fish were taken and electrocardiogram (ECG) was recorded.
Results and Conclusion- In bath method groups, the arterial blood oxygen in 2 mg/l group was significantly higher than the control group. In 10 mg/l group it was significantly lower than the control. CO2 and pH showed no significant difference between groups and controls. In injection groups, the arterial blood oxygen in 2 mg/kg, IV and 10 mg/kg, IV, was significantly lower than the control group. CO_2 in 6 and 8 mg/kg, IV group was significantly higher than the control but there was not any significant difference between CO_2 and pH in other experimental groups and control. Moreover, it was found that the average of heart rate in all groups of propofol anesthesia was significantly higher than the control groups ($p<0.05$) and in all groups in ECG, r to r distance (rr) has decreased. However, in ECG, no difference in the heart rate, rr, pr, qrs, qt distances was observed among all groups of recovery and the control.
Clinical Relevance- Results of this study can be used in anesthesia of fish for decreasing stress and movement at the time of surgery, diagnosis and in fish artificial fertilization procedure.
Key words- Anesthesia, Propofol, Blood gases, Grass carp, ECG**.**

[*] **Corresponding author:**
Rahim Peyghan, PhD
Department of Clinical Sciences, Faculty of Veterinary Medicine,
Shahid Chamran University of Ahvaz, Ahvaz, Iran.
E-mail address: : peyghan_r@scu.ac.ir

Introduction

Anesthesia in fish is used for decreasing stress and movement at the time of surgery, diagnosis and for fish transport. The most commonly used fish anaesthetic is tricaine methane sulfonate (MS_{222}). However, this anaesthetic is regarded as a carcinogenic and also a 21-day withdrawal period is required if the fish is intended for human consumption.[1] Propofol, as an anesthetic drug is well known in human and veterinary medicine. [2] However, there has been no report on its efficacy and the side effects when used on teloest fish. Propofol is a phenol derivative that was introduced in 1986 by Stuart pharmaceutical company. Other generic names of this drug are Diprivan, Kimofol and Pofol. This drug has a short half life in body (30-60 min) and is believed to affect the sodium and chloride channel in the CNS.[3,4] The anesthetic propofol is a potent enhancer of neuronal GABAergic currents and directly activates GABA currents. More recently, specific receptors have been postulated as important binding sites for propofol including a methionine in the third trans-membrane domain and a tyrosine with in the fourth transmembrane domain of the ß2 subunit. [5]
To date a number of studies have been done on the effects of propofol in human and terrestrial animals.[2,6,7] With regards to other anesthetic drugs, many investigations have been also done in fish.[8,9,10,11,12,13] But there is no report about the propofol anesthesia in grass carp and its side effects on the blood gases and ECG parameter of this fish species. Respectively this study aims to determine the efficacy of propofol and examines some physiologic impact of the drug in grass carp.

Materials and Methods

Fish
To carry out the experiment, 120 Grass carps (*Ctenopharyngodon idella*) weighting between 1-2 kg, supplied by one of private carp farm, Ahvaz-Iran, were chosen. After being transported to the laboratory in oxygenated water, they were stored in 12 large aquariums. The aquarium water was de-chlorinated tab water. The water, used in the experiments, came from the same source and had a mean temperature of 20-24 ̊C and was air saturated with respect to oxygen.

Experiments
Fishes were divided randomly into 12 groups of 10 fish each. Five groups were anesthetized by bath method with concentrations 2, 4, 6, 8 and 10 mg/l and 5 groups by injection method via caudal vein with doses 2, 4, 6, 8 and 10 mg/kg, IV. Normal saline was injected to 10 fish and this group was considered as the control injection group.

Sampling and Analysis
As for ECG recordings, we inserted three small clamps. ECG electrodes of electrocardiogram instrument (FX-120, Japan) were attached to the ventral surface of fish body: two electrodes lateral (one on each side) to the base of the heart and one electrode lateral to the midline near anus (Fig. 1).
For determination of stages, balance, respiration, caudal peduncle pinch (noxious stimuli), reactions to handling (touch stimuli) and swimming activity were observed and assessed during the entire experiment according to Graham, and Iwana, 1990.[11] Completely anaesthetized fish displayed no response to handling and the reflex reactions to the caudal peduncle pinch were absent, whereas recovered fish reacted to handling and swim normally.

Continuous ECG was recorded in different leads. The fish was oxygenated by freshwater with sustained flow across gills during the recording. After recording the electrocardiogram of all fishes, the average and standard deviation of the blood gases and electrocardiogram parameters (heart rates, pr and st segment, qrs, rr and tp distances) were calculated.
For blood gas analysis, at the time of anesthesia and recovery, arterial blood samples from dorsal aorta of fish were taken (Fig. 2). After transferring of blood in ice bag, blood gases and pH of all fish were measured immediately with Radiometer (ABL5, Denmark). Arterial blood was analyzed for oxygen tension (PaO2), pH, total carbon dioxide content of plasma. These parameters were measured with Radiometer set-up at 25°C. For conducting this experiment, the method details of Andersen and Wang (2002) was followed.

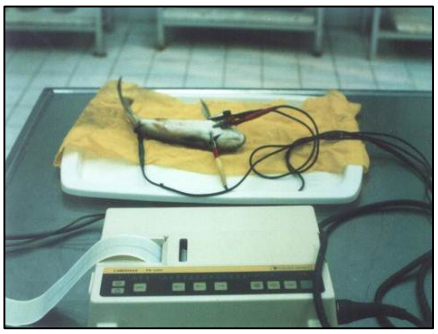

Figure 1. Ventral view of grass carp showing electrode placement.

Figure 2. Blood sampling from dorsal aorta of fish

Statistical Analysis
The means and standard deviation of the parameters were estimated and the data analysis was done through running one way analysis of variance using SPSS-11.5 software. The p values less than 0.05 were considered as significant.

Results

The results of data analysis revealed that, in bath methods the fish (100%) were successfully anesthetized and reached to stage 3, plane 2 in average time of 137±42 second without mortality in all concentrations (Table 1). In 6, 8 and 10 mg/l groups, the fishes reached to stage 3, plane 3 without mortality. In injection method in all doses the fish (100%) were successfully anesthetized and reached to stage 3, plane 3 (Table 2). However, in 8 mg/kg 1 fish (10%) and in 10 mg/l 2 fish (20%) reached to stage 3, plane 4 and did not recover.
The recovery time spans for concentrations of 2, 4, 6, 8 and 10 mg/l were 5, 16, 10, 31 and 12 min respectively. The recovery time spans for doses 2, 4, 6, 8 and 10 mg/kg were 9,6, 18, 18, 36 and 41 min respectively. As the results showed that recovery time in injection method was significantly higher than in the bath method.
According to results, in injection method in all concentrations the fish were successfully anesthetized and reached to stage 3, plane 3 in average time of 240±84 second without mortality. The detail of each group have been reported and summarized in table 2.

Table 1. Time to reach to anesthetic stage in bath method (sec.)

Treatment	Stage 1	Stage 2	Stage 3			Stage 4
			Plane 1	Plane 2	Plane 3	
2 mg/l	-*	-	72±20	137±42	-	-
4 mg/l	-	-	65±11.7	84±21	300±10	-
6 mg/l	-	-	38±14.9	56±23	124±22	-
8 mg/l	-	-	93±22	135±31	279±66	-
10 mg/l	-	-	15±7	22±7.5	74±13	-

*- No data in the box means that the stage sign was not detected or passed by the fish.

Table 2. Time to reach to anesthetic stage in injection method (sec.)

Treatment	Stage 1	Stage 2	Stage 3			Stage 4
			Plane 1	Plane 2	Plane 3	
2 mg/l	-*	-	160±34	180±60	240±84	-
4 mg/l	-	-	60±24	95±35	182±80	-
6 mg/l	-	-	-	112±15	195±30	-
8 mg/l	-	-	184±81	288±100	290±75	-
10 mg/l	-	-	-	84±32	107±17	-

*- No data in the box means that the stage sign was not detected or passed by the fish.

In bath groups, the arterial blood oxygen in 2 mg/l was significantly higher than the control group and in 10 mg/l group oxygen was significantly lower than the controls. Concerning CO2 and pH showed no significant difference was found between experimental groups and control group (Table 3). In injection groups, the arterial blood oxygen in anesthesia with 2 mg/kg and 10 mg/kg propofol was significantly lower than the control group (Table 4). However, in 6 and 8 mg/kg the arterial blood oxygen was significantly higher than the control. In injection groups CO_2 in 6 and 8 mg/kg group was significantly higher than the control but there was not any significant difference between CO_2 and pH in other experimental groups and control.

Table 3. Blood gas and pH changes in anesthesia with propofol in bath method.

Concentration mg/l	CO2		O2		pH	
	Anesthesia	Recovery	Anesthesia	Recovery	Anesthesia	Recovery
2	11.1±1.4	11±1.4	11.3±3.7*	13.4±3.8*	7.5±0.1	7.5±0.07
4	9.5±1.6	11.6±0.79	6±1	6.7±3.4	7.5±0.15	7.5±0.05
6	11.1±1.6	9.7±1.9	3±1.1	7.9±2.6	7.5±0.15	7.4±0.18
8	9.7±1.2	13.3±0.9	6.8±3.6	6.1±2.7	7.5±0.07	7.6±0.05
10	10.5±1	10.4±0.54	2.4±1.5*	3.6±1.1*	7.5±0.09	7.6±0.05
Control	8.1±2.9		5.5±2.9*		7.6±0.16	

*- Significantly different from control ($p < 0.05$).

Table 4. Blood gas and pH changes in anesthesia with propofol in injection method.

Dose mg/kg	CO2		O2		pH	
	Anesthesia	Recovery	Anesthesia	Recovery	Anesthesia	Recovery
2	10.2±0.9	11±1.4	3.6±2.4*	6±1.7	7.6±0.04	7.6±0.1
4	12.3±2.7	12.6±0.79	5±4.2	6.7±3.4	7.5±0.08	7.5±0.08
6	14.3±1.5*	11.7±1.9	5.7±2.8	7.9±2.6	7.7±0.05	7.5±0.05
8	13.3±1.9*	12.3±0.9	6.2±1.2	7.7±2.6	7.6±0.01	7.5±0.1
10	11.9±1.1	11.4±0.54	3.2±2.3*	5.1±2.6	7.6±0.05	7.5±0.1
Control	8.1±2.9*		5.5±2.9*		7.6±0.16	

*- Significantly different from control ($p < 0.05$).

In the ECG study in control group, the average of pr, qt, qrs and rr segments were 0.1±0.02, 0.41±0.03, 0.04±0.01, 8.9±5.5 second respectively. The mean heart rate of control group was 9.8±6.1 beat per min. In anesthesia groups there was no significant difference between themselves but the heart rates in these groups were significantly higher than the control groups. However, the tp and rr segments in experimental groups were lower than the control. No significant difference in the heart rate, rr, pr, qrs, qt distances was observed between recovery and control groups.

Discussion

The first part of this study was focused on assessing the efficacy of 5 different concentration of propofol as a new anesthetic in fish to establish a minimum dose producing optimal anesthetic state. In bath method in all concentration and in injection method in doses of 2, 4 and 6 mg/kg the fishes (100%) were successfully anesthetized. In shark, in an experiment propofol (2.5 mg/kg) was administered over 30 sec via the caudal vein, heart rate and respiratory rate did not change significantly over time. The righting response returned within 60 min in 44% of the sharks, 75 min in 22% of the sharks, and over 200 min in 33% of the sharks. All sharks recovered uneventfully and Propofol provided a safe anesthetic event for spotted bamboo sharks.[14] Parenteral anesthetic protocols for short-term immobilization were evaluated in twenty 4-yr-old Gulf of Mexico sturgeon (*Acipenser oxyrinchus de soti*). An initial dose-response trial determined the efficacy of either propofol (3.5-7.5 mg/kg, i.v.) in this fish species at the dosages used in that study, propofol effectively induced a light plane of anesthesia.[15] Therefore, using these concentrations and doses can be recommended in aquaculture; but, economically speaking, the best doses can be 4 mg/l in bath method and 2

mg/kg by injection that was the minimum dose which producing optimal anesthetic state for each method respectively.

The second part of this study was to examine the effects of anesthesia on blood gas, pH and ECG of the fish. Changes in blood and heart are the most important changes in body during anesthesia. According to literature the most important factors affecting the fish blood parameters are: age, diet, drugs and diseases.[16]

In the present study, the heart rate in anesthetized group was relatively high, when compared to control group fish. This suggests an effect on the heart by exposure to hypoxia. In teleost fish, splenic contraction is the primary cause of arterial blood oxygen increase during hypoxaemia induced elevation of circulating catecholamines.[18] The immediate rise in heart rate following anaesthesia may well be due to high plasma catecholamine concentrations. Cardiovascular function is changed when teleost fish are anaesthetized. Changes include direct effects of the anaesthetics on tissues such as heart muscle, the smooth muscle in vascular walls, and autonomic nerves as well as secondary effects of the associated hypoxia and acid–base disturbances.[17]

We showed that the ECG pattern and duration of qrs, qt and qt were not influenced by propofol induced anesthesia. However the results of Cotter and Rodnick, 2006, indicated that clove oil and tricaine exerted some effects on heart rate (HR), heart rate variability (HRV), QT interval, QRS duration, and QRS amplitude in rainbow trout.[18] Conversely, benzocaine-anesthetized trout exhibited HR oscillations and QRS amplitude variations not found in clove oil- or tricaine-exposed trout. These differences may be due to different mechanisms of action of these drugs. For example, clove oil and its chief component, eugenol, are known to affect cell membrane channels in a tissue-specific manner. For instance, eugenol activates Ca2+ channels in rat dorsal root ganglion cells [18] and inhibits Ca2+ and K+ channels in guinea pig cardiac muscle.[20]

Findings of the study indicated that anesthesia with propofol has no marked effect on heart activities, blood gases and pH. The result showed that the changes had not a direct correlation with the propofol concentration. Although grass carp had showed a decrease in oxygen at the 2 mg/kg, 10 mg/kg, 2 mg/l and 10 mg/l concentrations, but oxygen had remained relatively stable throughout the other concentrations or elevated significantly. These changes can be due to depression of the central nervous system. Depression of the CNS inhibits ventilation and can induce hypoxaemia. In most teleostean species hypoxia elicits a significant increase in both heart rate and respiratory rate that can result in compensatory elevation of blood oxygen level.[21]

In bath method in all concentration the fish were successfully anesthetized and in injection method in doses of 2, 4 and 6 mg/kg were also successfully anesthetized. Therefore using these concentrations of propofol can be recommended in aquaculture but economically the best concentration in bath method and injection seems to be 4 mg/l and 2 mg/kg respectively. We suggest that propofol can be considered as a comparatively safe anesthetic drug with little side effects on blood parameter and heart; nevertheless, for introduction of this product for use in aquaculture, more investigation should be done on pathology and other side effects of this drug in fish.

References

1. Pirhonen, J. and Schreck, C.B. Effects of anaesthesia with MS-222, clove oil and CO2 on feed intake and plasma cortisol in steelhead trout (*Oncorhynchus mykiss*). *Aquaculture*. 2003; 220. 507–514.

2. Yamaky, F. and Perriera, G. Effects of propofol on the electrocardiogram and blood pressure of clinically healthy cats. *World Small Anim Anesthesia*. 2001; 22. 35-42.
3. Glen, JB, Hanter, SC and Wood, P. Interaction studies and other investigation of the pharmacology of propofol. *Postgrad Med J.* 1985; 61. 2. 7-14.
4. Sebel, P. and Lowdon, J. Propofol: A new intravenous anesthetic. *Anesthesiology*. 1989; 71. 260-277.
5. Watt, EE., Betts, BA., Kotey, FO., Humbert, DJ., Griffith, TN., Kelly, EW., Veneskey, KC., Gill, N., Rowan, KC., Jenkins, A. and Hall, AC. Menthol shares general anesthetic activity and sites of action on the GABAA receptor with the intravenous agent, propofol. *Europ J Pharma*. 2008; 590. 120–126.
6. Nagashiyama, Y. and Furkawa, Y. Propofol increase contractility of isolated blood-perfused left ventricular muscle in the dog. *J Anesth*. 2000; 14. 45-47.
7. Goodchild, C. and Serrao, J. Cardiovascular effect of propofol in the anaesthetized dog. *Brit J Anesthesia*. 1989; 63. 87-92.
8. Aissaus, A, Altimiros, J and Tort, L Cardiac conduction times in *Sparus auratus* at different heart rates. *J Fish Biol*, 1998; 52. 1154-1164.
9. Bruecker, P and Graham. M, The effects of the anesthetic ketamine hydrocholoride on oxygen consumption rates and behavior in the fish *Hores citrinellam*. *Comp Biochem Physiol*. 1993; 104 (1). 57-59.
10. Ferreire, JT, Smit, GL and Schoonbee, HJ, The effect of the anesthetic benzocaine hydrochloride on red cell fragilities in C*yprinus carpio. J Fish Biol*, (1981); 18 (2). 123-126.
11. Graham, MS and Iwana, GK The physiologic effects of the anesthetic ketamine hydrochloride on two salmonid species. *Aquacultre*. 1990; 90 (3-4). 323-331.
12. Hill, JV, Davison, W. and Forster, ME The effects of fish anaesthetics (MS222, metomidate, and AQUI-S) on heart ventricle, the cardiac vagus and branchial vessels from Chinook salmon (*Oncorhynchus tshawytscha*). *Fish Physiol Biochem*. 2004; 27, 19–28.
13. Palić, D., Herolt, D.M., Andreasen, C.B., Menzel, B.W. and Roth, J.A. Anesthetic efficacy of tricaine methanesulfonate, metomidate and eugenol: Effects on plasma cortisol concentration and neutrophil function in fathead minnows *(Pimephales promelas* Rafinesque, 1820). *Aquaculture*. 2006; 254. 675–685.
14. Miller, SM, Mitchell, MA, Heatley, JJ Wolf, T., Lapuz, F., Lafortune, M., and Smith, JA, Clinical and Cardio respiratory effects of Propofol in the spotted bamboo shark (*Chyllosyllium pligosum*). *J Zoo Wildlife Med*. 2005; 36 (4): 673-676.
15. Fleming, GJ, Heard, DJ, Floyd, RF and Riggs, A. Evaluation of Propofol and Medetomidine-Ketamine for Short-Term Immobilization of Gulf of Mexico Sturgeon (*Acipenser oxyrinchus de soti*). *J Zoo Wildlife Med*. 2003; 34 (2):153-8.
16. Booth, NH and McDonald. E *Veterinary pharmacology and Therapeutics*. 6th ed. State University Press, Ames, Iowa, 165-167, 253-265. 1988.
17. Rothwell, SE, Black, SE., Jerrett, AR. and Forster, ME. Cardiovascular changes and catecholamine release following anaesthesia in Chinook salmon (*Oncorhynchus tshawytscha*) and snapper (*Pagrus auratus*). *Comp Biochem Physiol,* Part A. 2005; 140. 289– 298.
18. Okhubo, T., Kitamura, K., Eugenol activates Ca2+-permeable currents in rat dorsal root ganglion cells. *J Dentis Res*. 1997; 76, 1737–1744.

19. Cotter, PA and Rodnick, KJ Differential effects of anesthetics on electrical properties of the rainbow trout (*Oncorhynchus mykiss*) heart. *Comp Biochem Physiol*, Part A, 2006; 145. 158-165.
20. Sensch, O., Vierling, W., Brandt, W., Reiter, W. Effects of inhibition of calcium and potassium currents in guinea-pig cardiac contraction: comparison of beta-caryophyllene oxide, eugenol, and nifedipine. *Brit J Pharma*. 2000; 131, 1089–1096.
21. Andersen, JB and Wang, T Effects of anaesthesia on blood gases, acid–base status and ions in the toad *Bufo marinus. Comp Biochem Physiol*, Part A. 2002; 131. 639–646.

چکیده

بررسی بی هوشی ماهی کپور علفخوار با داروی پروپوفول و تاثیر آن بر الکتروکاردیوگرام، گازهای خونی و pH خون

رحیم پیغان[1]، احمد علی پاپهن[1]، هادی نداف[1]، احمد عبادی[2]

[1] گروه علوم درمانگاهی، دانشکده دامپزشکی دانشگاه شهید چمران، اهواز، ایران،
[2] دانشگاه علوم پزشکی جندی شاپور، اهواز، ایران.

هدف- بررسی امکان استفاده از پروپوفول در بیهوشی ماهی کپور علفخوار و تاثیر آن بر فعالیت های الکتریکی قلب، گازهای خونی و pH خون.

طرح مطالعه - مطالعه تجربی بر موجود زنده.

حیوانات - ۱۲۰ قطعه ماهی کپور علفخوار (آمور) با محدوده وزنی ۱-۲ کیلوگرم که به ظاهر سالم بودند.

روش کار- ماهی‌ها به صورت تصادفی به ۱۲ گروه ۱۰ تایی تقسیم شدند. دو گروه به عنوان شاهد در نظر گرفته شد. پنج گروه به ترتیب با مقادیر ۲ ، ۴ ، ۶ ، ۸ و ۱۰ میلی‌گرم در لیتر پروپوفول به روش محلول در آب و پنج گروه با مقادیر ۲ ، ۴ ، ۶ ، ۸ و ۱۰ میلی‌گرم در کیلوگرم به روش تزریق داخل وریدی بی‌هوش گردیدند. ماهیان هر گروه پس از بیهوشی و بعد از بهبودی از آب خارج شده و الکتروکاردیوگرام آنها ثبت و از شریان آئورت پشتی خونگیری می گردید.

نتایج و نتیجه گیری- در روش تزریقی این زمان به ترتیب ۹،۶ ۱۸، ۱۸، ۳۶ و ۴۱ دقیقه بوده است. در روش غوطه وری بیشترین میزان اکسیژن شریانی در گروه ۲ میلی گرم در لیتر بوده است که نسبت به گروه شاهد افزایش معنی داری را نشان می دهد. فشار اکسیژن شریانی در گروه ۱۰ میلی گرم در لیتر نیز به طور معنی داری از گروه شاهد کمتر بوده است. فشار دی اکسید کربن و پ-اچ در گروههای مورد مطالعه و گروه شاهد نیز تفاوت معنی داری نشان نمی دهد. در روش تزریقی بیشترین میزان اکسیژن شریانی در گروه ۲ میلی گرم در کیلوگرم و ۱۰ میلی گرم در کیلوگرم بوده است. در روش تزریقی میزان دی اکسید کربن خون شریانی در میزان های ۶ و ۸ میلی گرم در کیلوگرم به طور معنی داری از گروه شاهد بیشتر بوده است اما پ-اچ خون و دیگر موارد مقایسه شده تفاوت معنی داری نداشته است. در گروههای بیهوشی متوسط ضربان قلب بطور معنی داری از گروه شاهد بیشتر بوده است و فاصله rr نیز کاهش معنی داری را نشان می دهد ($p < ۰/۰۵$). اما در فواصل qt ، qrs و pr با گروه شاهد، تفاوت معنی دار نبوده است ($p > ۰/۰۵$).

کاربرد بالینی- یافته های این مطالعه در بیهوشی ماهی به منظور جراحی ماهی، انجام عملیات تکثیر مصنوعی و بررسی های تشخیصی مورد استفاده قرار می گیرد.

کلید واژگان- بیهوشی، ماهی کپور علفخوار، پروپوفول، گازهای خونی، الکتروکاردیوگرام.