African Journal of Biotechnology Vol. 11(22), pp. 6149-6142, 15 March, 2012
Available online at http://www.academicjournals.org/AJB
DOI: 10.5897/AJB11.1639
ISSN 1684–5315 © 2012 Academic Journals

Full Length Research Paper

Comparison of clinical hematological changes under anesthetization in Crucian carp (Carassius auratus auratus) following treatment with local anesthetics

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Accepted 17 February, 2012

The objectives of this study were to compare the clinical hematological changes under anesthetization in Crucian carp (Carassius auratus auratus) due to treatment with local anesthetics. Our data indicate that the values declined significantly ($P < 0.05$) with dissolved oxygen amount after anesthetization, furthermore, the values increased significantly ($P < 0.05$) with blood carbon dioxide amount after anesthetization with local anesthetic. The values of platelet (PLT), red blood cell distribution width coefficient of variation (RDW-CV) significantly declined ($P < 0.05$) white blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC). Red blood cell distribution width standard deviation (RDW-SD), plateletcrit (PCT), mean platelet volume (MPV) significantly increased ($P < 0.05$) but PLT values dropped to 30% of the originally worth with local anesthetics.

Key words: Blood property tests, carbon dioxide amount, Crucian carp, dissolved oxygen amount, local anesthetics.

INTRODUCTION

The science and technology of fish have also improved much (Yueh et al., 2005; Twan et al., 2006; Tomy et al., 2007). Human local anesthetics are divided into two classes in accordance with chemical constitution: The derivatives of esters, like tetracaine, procaine, etc., the derivatives of amides, like lidocaine, mepivacaine, etc., are the anesthetics which human medical circles and academia often use at present (King, 2006). Human local anesthetics is called conductive anesthesia. They are most suitably used for anesthetizing the living beings of the aquatic region because they can be fast conducted by water. Therefore, they can anesthetize the living beings in the aquatic region (King, 2006). And hematology studies will be a valuable tool for prognosis and primary diagnosis in some infectious diseases. So the aims of this study were to analyze and compare the clinical hematological changes under anesthetization in Crucian carp (Carassius auratus auratus) due to the treatment with human local anesthetics. These data and statistical results could be a useful database for prognosis and primary diagnosis in some infectious diseases of fish.

The inspection of the blood of beast in medical circles has already known that there are rises in dogs, the cat, the horse, the ox, the sheep, the chicken, the duck, etc. in recent years (Ha, 2006). These animals' blood are checked and remembered automatically by the human general blood property test value electron measurement. The measurement values of blood property are extremely accurate. Domestic veterinarians all regard as the basis

Abbreviations: PLT, Platelet; **RDW-CV,** red blood cell distribution width coefficient of variation; **WBC,** white blood cells; **RBC,** red blood cells; **HGB,** hemoglobin; **HCT,** haematocrit; **MCV,** mean corpuscular volume; **MCH,** mean corpuscular hemoglobin; **MCHC,** mean corpuscular hemoglobin concentration; **RDW-SD,** red blood cell distribution width standard deviation; **PCT,** plateletcrit; **MPV,** mean platelet volume.

data of diagnosing, at present (Ha, 2006). Lidocaine and tetracaine were used in this research. While carrying out the experiment, obtaining dissolved oxygen amounts, carbon dioxide amounts and blood property test values before and after anesthetization a teleost blood with the two anesthetics, and analyze these values with non-parametric statistical methods.

MATERIALS AND METHODS

Acclimation of Crucian carp

Six fishes (average weight about (334.7 ± 0.3 g)) was raised for 21 days in 50 L barrels, plating warm equipment in the barrel and feeding fish with the commercial eel's fodder while raising and acclimatizing them, and about 10 for every morning. The remaining fodder and fish excrement were artificially collected on the last day to avoid pollution of water. Water temperature was about 25°C for 21 days. Feeding was stopped more than 24 h before the experiment began (Gilderhus et al., 1991).

Anesthetics and anesthesia of the Crucian carp

Powders of Lidocaine and tetracaine are pure raw materials (not including HCL), (Lidocaine and tetracaine powders, 100 g in one pot) made by Sigma chemical raw materials manufacturing company. Lidocaine and tetracaine are difficult to dissolve in water, so dissolve them in 95% alcohol (an extremely small amount), 20 mg/ml each times with lidocaine, and 5 mg/ml each time with tetracaine. The anesthetization barrel with 5 L of fresh water contains only one anesthetic for anesthetizing. One anesthetic was offered each for 3 to 5 min per interval as these fish present the state of total overbalancing (3B) (Mattson and Riple., 1989). When the experiment begins, six fish were grabbed from the raising barrel randomly. The experiment starts by obtaining dissolved oxygen amounts and blood property test values with the two anesthetics before and after anesthetization of Crucian carp, and then by nonparametric statistical method to analyze.

Fish's blood oxygen amount were analyzed before and after anesthetization

The domesticated six fishes in the barrel were picked at random and blood was collected into heparin sodium injection apparatus [needles (18G ×$1\frac{1}{2}$") injection and syringe of 10 ml].Small artery blood from the vertebra tail of fish body was retrieved. Two treatments of blood with and without were added by the two anesthetics and then poured into the adopt blood vessel containing heparin injection solution, and shake artificially evenly. The solution was put into ice water immediately and then put into the instrument namely AVL OMNI to analyze the dissolved oxygen amount and carbon dioxide amount immediately.

Fish's blood properties were analyzed before and after anesthetization

First, the domesticated fishes in the barrel were picked and raised at random. The blood sample was collected into heparin sodium injection apparatus [needles (18G ×$1\frac{1}{2}$") injection and syringe of 10 ml]. Small artery blood from the vertebra tail of fish body was retrieved. Two treatments of blood with and without were added by the two anesthetics and then poured into the adopted blood vessel containing heparin injection solution, shake artificially evenly. The solution was put into the instrument namely SYSMEX SE-9000 to be analyzed for general blood properties.

RESULTS AND DISCUSSION

Anesthetic security analysis

All fishes resumed balance and no fatalities appeared within 5 min. The two powdery local anesthetics did not give out any smell with high security for users.

Analysis of the amount of blood dissolved oxygen

Fish anaesthetized was kept in touch with insufficient gas and these fishes lack large amounts of oxygen after anesthetization with the two anesthetics. The blood oxygen pressure before anesthetization dropped at greater disparity. Anaesthetizing Crucian carp time should not be too long, and Crucian carp organization necrosis should be avoided because of long duration of insufficiency of oxygen. The values of blood oxygen pressure (PO_2) are listed before and after anesthetization (Table 1). They decreased significantly ($P < 0.05$) according to Sign-test. The values of blood carbon dioxide amount (PCO_2) are listed before and after anesthetization (Table 1) and they increased significantly ($P<0.05$) according to Sign-test.

Blood properties examined and analyzed

Anesthetizing by tetracaine and lidocaine, the changes of blood property were tested: White blood cells (WBC), red blood cells (RBC), hemoglobin (HGB), hematocrit (HCT), mean corpuscular volume (MCV), mean corpuscular hemoglobin (MCH) , mean corpuscular hemoglobin concentration (MCHC), red blood cell distribution width standard deviation (RDW-SD), plateletcrit (PCT), platelet (PLT), red blood cell distribution width coefficient of variation (RDW-CV), mean platelet volume(MPV) (Tables 2 and 3); Pass Sign-test, $P < 0.05$, have significant differences ($P = 0.05$). It showed that Crucian carp blood property test values significantly changed before and after anesthetization; among them multiple blood property test values were all on the rise after anesthetization. Only PLT and RDW-CV presented the downward trend, and the PLT values dropped to 30% of the original value (Tables 2 and 3).

PLT is the value for calculating the total number of blood platelets, PLT largely reduced after anesthetization, and the blood platelet generally have the following functions to fish's body: Keeping the fullness of the blood capillary wall; they gather and stop the thrombus to protect together promptly when the blood vessel is

Table 1. Blood test of oxygen pressure (PO_2) and carbon dioxide pressure (PCO_2) level in tetracaine or lidocaine treatment Carassius auratus auratus.

Treatment (mmHg)	Tetracaine		Lidocaine	
	b	a	b	a
PO_2	18.35 ± 2.9	2.63 ± 1.9	25.47 ± 3.6	9.95 ± 2.25
PCO_2	45.37 ± 1.85	76.45 ± 3.95	45.5 ± 2.25	55.1 ± 2.45

a: After anesthetization, b: Before anesthetization.

Table 2. Blood test of oxygen pressure (PO_2) and carbon dioxide pressure (PCO_2) level in tetracaine or lidocaine treatment Carassius auratus auratus.

Treatment (mmHg)	Tetracaine		Lidocaine	
	b	a	b	a
PO_2	18.35 ± 2.9	2.63 ± 1.9	25.47 ± 3.6	9.95 ± 2.25
PCO_2	45.37 ± 1.85	76.45 ± 3.95	45.5 ± 2.25	55.1 ± 2.45

a: After anesthetization, b: Before anesthetization.

Table 3. Blood property test of tetracaine treatment Carassius auratus auratus.

Item	Before *lidocaine treatment*	After *lidocaine treatment*
WBC	*971.387± 30.84 10^3/μl*	*973.28 ± 30.49 10^3/μl*
RBC	*1.54 ± 0.13 10^6/*μl	*1.70 ± 0.13 10^6/μl*
HGB	*8.50 ± 0.70 g/dl*	*10.75 ± 1.10 g/dl*
HCT	*23.45 ± 3.15 %*	*27.92 ± 3.05 %*
MCV	*163.22 ± 2.45 fL*	*165.75 ± 2.80 fL*
MCH	*53.42 ± 1.35 pg*	*55.15 ± 1.65 pg*
MCHC	*34.05 ± 1.4 g/dL*	*34.65 ± 1.40 g/dL*
PLT	*122.33 ± 11.5 10^3/μl*	*35.17 ± 3.50 10^3/μl*
RDW-SD	*45.48 ± 5.15 fL*	*47.80 ± 4.80 fL*
RDW-CV	*12.18 ± 0.95 %*	*8.27 ± 0.50 %*
MPV	*9.72 ± 0.85 fL*	*11.73 ± 0.20 fL*
PCT	*0.05 ± 0.02 %*	*0.07 ± 0.02 %*

WBC: White blood cell count; RBC: Red blood cell count; HGB: Hemoglobin; HCT: Hematocrit; MCV: Mean corpuscular volume; MCH: Mean corpuscular hemoglobin; MCHC: Mean corpuscular hemoglobin concentration; PLT: Platelet; PDW: Platelet distribution width; MPV:Mean platelet volume; RDW–CV: (1SD/MCV) *100 (SD: standard deviation).

damaged; control solidify function and clot to shrink; the curing of the wound; oppose the forming of inflammation; view blood platelet functions, after anesthetization; wound the fish's body for collecting fish's blood by needle, because PLT reduces sharply, it may cause the wound not to heal and the fish to die. The point can be offered to the academic researcher by collecting fish's blood by needle to examine after anaesthetization of teleost, as a security reference index.

The numbers of leukocyte (WBC) (leukocyte is the main immune system of fish's body) increase after anesthetization showed the unexpected changes of the living water environments or invasion of outside material; the numbers of red blood cell (RBC), generally speaking, the factors influencing red blood cell are at the age or the weight, sex, season, and temperature, etc. (Wu et al., 2000). But the increase of RBC after anesthetization show that fish's body is kept in touch with insufficient gas when anesthetizing. The oxygen is scarce, so RBC increases for carrying more oxygen to every place in the body, to keep the basic physiological function. HGB is the amount of hemoglobin, an effective index of taking oxygen ability in the blood caused an increased show with little oxygen amount after anesthetization, so HGB increased, for obtaining more oxygen; the average volume value (MCV) with a red blood cell increases after

anesthetization and show that dissolved oxygen amount decrease in fish's blood. So MCV is strengthened by holding more oxygen, and keeping the basic physiological function to fish's body; the average concentration of hemoglobin of red blood cells (MCHC) increase after anesthetization and show that dissolved oxygen amount is decreasing in fish's blood, so MCHC increase for combining more oxygen.

Conclusion

From this research, Crucian carp blood dissolved oxygen amount and blood carbon dioxide amount before and after anesthetization have significant differences. PLT and RDW-CV values significantly decline ($P < 0.05$). The values of WBC, RBC, HGB, HCT, MCV, MCH and MCHC, significantly increase ($P < 0.05$) and PLT value dropped to 30% of its original value. This study could be recommended for monitoring and surveillance programs in health status in teleost.

REFERENCES

Gilderhus PA, Lemm CA, Wods LC (1991) Benzocaine as an anesthetic for striped bass. Prog. Fish. Cult. 53:105-107.

Ha MF (2006) Hematology Ho-Chi Book Publishing CO. Ltd. 26; 701-703.

Jeng SR, Yeun WS, Chen GR, Lee YH, Dufour S, Chang CF (2007) Differential expression and regulation of gonadotropins and their receptors in the Japanese eel, Anguilla japonica. Gen. Comp. Endocrinol. 154:161-173.

King HG (2006) The rinciples of Human Anesthesia, Yi-Hsien Publishing CO., Ltd, pp. 193-194.

Mattson NS, Ripple TH (1989) Metomidate, a better anesthetic for cod (*Gadus morhua*) in comparison with benzocaine, MS-222, chlorobutanol, and phenoxyethanol. Aquaculture, 83: 89-94.

Tomy S, Wu GC, Huang HR, Dufour S, Chang CF (2007.) Developmental expression of key steroidogenic enzymes in the brain of protandrous black porgy fish, Acanthopagrus schlegel. J. Neuroendocrinol. 19: 643-655.

Twan WH, Hwang JS, Lee YH, Wu HF, Tung YS, Chang CF (2006). Hormones and reproduction in scleractinian corals. Comp. Biochem. Physiol. Part A, 144: 247-253.

Wu FC, Chen ML, Ting YY (2006) Hematological analysis in cultured healthy mullet. (*Mugil cephalus*) Bull. Tainan Fish Cult. Stn. TFRI. 4: 27-36.

Yueh WS, Thomas P, Chang CF (2005) 21-Trihydroxy-4-pregnen-3-one as an oocyte maturation-inducing stero.id in protandrous black porgy, *Acanthopagrus schlegeli*. Gen. Comp. Endocrinol. 140: 184-191.