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Effects of local anesthetics in Fish

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Abstract

Detection of Fish blood was prevalent used in fisheries management and disease diagnosis. Anesthesia is often used before phlebotomize in fish. The aim of this study was to compared and analyzed hormones level before and after local anesthetics Tilapia. Two anesthetic, lidocaine and tetracaine, were used in this study. After anesthetization the level of triiodothyronine (T3) and thyroxine(T4) were significantly increased ($P < 0.05$) by nonparametric statistical methods tested in tilapia blood. However, luteinizing hormone(LH), follicle stimulating hormine (FSH)were not significantly change after anesthetization($P < 0.05$). In addition, White blood cells (WBC), Red blood cells (RBC), Mean corpuscular volume (MVC), Mean corpuscular hemoglobin(MCH), Mean corpuscular hemoglobin concentration (MCHC), Red blood cell distribution width standard deviation (RDW-SD) were also no change($P < 0.05$). The Hemoglobin (HGB) and Hematocrit (HCT) were significant rising($P < 0.05$) and Platelet (PLT)was significant declined ($P < 0.05$) after anesthetization. These results in this study reveal that anesthetic can effect blood property and hormone values in fish.

Key words:Tilapia, triiodothyronine, thyroxine, fish hormones

局部麻醉劑麻醉魚類的效應

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摘要

魚類血液檢測時常被應用在魚類的培育管理與疾病診斷上。在本研究中作者用統計方法分析和比較吳郭魚麻醉前後, 血中荷爾蒙數值及血液性狀指數值的變化。吳郭魚麻醉後血中的三碘甲狀腺素(T3, triiodothyronine) 和甲狀腺素(T4, thyroxine)含量有明顯的提升($P<0.05$)，不過黃體素(LH, luteinizing hormone) 和促卵泡激素(FSH, follicle stimulating hormone)則沒有變化($P<0.05$)。此外 RDW-CV，白血球(White blood cells, WBC)、紅血球(Red blood cells, RBC)、平均紅血球體積(Mean corpuscular volume, MCV)、平均紅血球血紅素濃度(Mean corpuscular hemoglobin concentration, MCHC)、平均紅血球血紅素量(Mean corpuscular hemoglobin, MCH)、紅血球體積分布寬度標準差(Red blood cell distribution width standard deviation, RDW-SD)、血小板體積(Plateletcrit,

PCT)、平均血小板體積(Mean platelet volume ,MPV)也沒有明顯變化(P<0.05)。血紅素(Hemoglobin ,HGB)、血容積比(Hematocrit,HCT)顯著性上升(P＜0.05)且血小板(Platelet,PLT)值顯著性下降(P＜0.05)。這個研究顯現出麻醉劑影響魚類血液性狀指數及賀爾蒙值。

關鍵詞：吳郭魚、三碘甲狀腺素、甲狀腺素、魚類荷爾蒙。

Introduction

Thyroid hormones, i.e., thyroxine (T4) and triiodothyronine (T3), play an important role in the regulation of metabolism, reproduction, growth, and development in vertebrates (Walpita et al., 2009), including fish (Liu et al., 2000). Similar to mammals (Yu et al., 2010), thyroid hormone synthesis of teleosts is also regulated by feedback regulation through the hypothalamus–pituitary–thyroid (HPT) axis. The regulation of thyroid hormone homeostasis involves multiple steps, including iodine uptake, thyroid hormone synthesis, transport, deiodination, and binding to thyroid hormone receptors. Therefore, compounds that interact with any of these steps may interrupt the balance of thyroid hormones.

In both sexes, Luteinizing Hormone (LH) stimulates secretion of sex steroids from the gonads. In the testes, LH binds to receptors on Leydig cells, stimulating synthesis and secretion of testosterone (Costa et al., 2010). Theca cells in the ovary respond to LH stimulation by secretion of testosterone, which is converted into estrogen by adjacent granulosa cells. In females, ovulation of mature follicles on the ovary is induced by a large burst of LH secretion known as the preovulatory LH surge. Residual cells within ovulated follicles proliferate to form corpora lutea, which secrete the steroid hormones progesterone and estradiol. Progesterone is necessary for maintenance of pregnancy, and, in most mammals, LH is required for continued development and function of corpora lutea. The name luteinizing hormone derives from this effect of inducing luteinization of ovarian follicles.

As its name implies, Follicle-Stimulating Hormone (FSH) stimulates the maturation of ovarian follicles. Administration of FSH to humans and animals induces "superovulation", or development of more than the usual number of mature follicles and hence, an increased number of mature gametes. FSH is also critical for sperm production. It supports the function of Sertoli cells, which in turn support many aspects of sperm cell maturation.

In this study, we detected of the amounts of T3, T4, LH and FSH after anesthesia in Tilapia fish. The level of T3 and T4 will be affected by anesthesia treatment in Tilapia fish.

RESULTS AND DISCUSSION

Anesthetics security analysis

All fishes resumed balance and there was no death to appear within 5 minutes. As the two powdery local-anesthetics will not distribute any smell, the security was very high to the user.

Analysis and comparison of the levels of hormones in fish blood

Hormones would be metabolized and dissolution continuously that hormone must loose its activation by itself, must separate the serum to use as soon as possible after obtaining fish's body blood. The values of luteinizing hormone and follicle stimulating hormone of the Tilapia before and after anesthetization were listed with the two anesthetics (Table 1 and 2). There was no significant difference after sign-test ($P > 0.001$) (Yen, 2006). The values of triiodothyronine (T3) and thyroxine (T4) were listed before and after anesthetization (Table 3 and 4). After sign-test, the values of triiodothyronine and thyroxine before and after anaesthetization had significant difference with lidocaine and tetracaine.($P < 0.001$) Triiodothyronine values and thyroxine values were also significantly different between lidocaine and tetracaine (Fig. 1). After the Wilcoxon grade test, (Yen, 2006) there were significant difference ($P < 0.001$). Furthermore, Adrenocorticotropic hormone (ACTH), growth hormone (GH) and insulin were examined for many times in this research, but the amounts were smaller than the limitation of detection.

Analyses of blood properties examined and analyzed

Anesthetizing by tetracaine and lidocaine, the changes of blood property were analyzed including: 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 5 and 6); Pass Sign-test, $P < 0.05$, have significant differences. 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 70% of the original value (Tables 5 and 6).

PLT is the value for calculating the total number of blood platelets, PLT largely reduced after anesthetization, and the blood platelets generally have the following functions to fish's body: Keeping the fullness of the blood capillary wall; gathering and stopping the thrombus to protect together promptly when the blood vessel is damaged; controlling solidify function and clot to shrink; the curing of the wound; oppose the forming of inflammation; 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 increasing numbers of leukocyte (WBC) (leukocyte is the main immune system of fish's body) 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 showed 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 physio logical function to fish's body; the average concentration of hemoglobin of red blood cells (MCHC) increased after anesthetization and show that dissolved oxygen amount is decreasing in fish's blood, so MCHC increased forcombining more oxygen.

MATERIALS AND METHODS

Acclimation of tilapia

Healthy tilapia (*Oreochromis mossambicus*) (average weight : 85.7±0.3g) had been raised for twenty-one days in 50 liters barrel, took a warm equipment in the barrel, fed with the commercial eel's fodder, and about 10 for every morning, collected fodder remained and fish's excrement of the twenty-one days artificially, to avoid pollution of water, and the water temperature was about 25℃ in twenty -one days. Stopped feeding for more than 24 hours before the experiment begins (Gilderhus et al., 1991).

Anesthetics and anesthesia of the three fishes

Powders of lidocaine and tetracaine were pure raw materials (made by Sigma chemical company, concentration: 99.5%, not include Hcl), (Lidocaine and tetracaine powders, 100g in one pot). Lidocaine and tetracaine were difficult to dissolve in water, so dissolved them with 95% alcohol extremely a small amount, 20 mg/ml each times of lidocaine, 5 mg/ml each times of tetracaine. Put into the anesthetization barrel with 5 liters of fresh water only containing one anesthetic for anesthetizing, offered each one anesthetic for 3-5 minutes of interval each times, as these fishes presented the state of totally overbalancing (3B) (Mattson and Riple., 1989).

Obtaining hormones value before and after anesthetization

While the experiment startted, six fishes were grabbed from raising barrels to the two anesthetics barrels at randomly. Obtained hormones amount with the two anesthetics before and after anesthetization of Common carp, Crucian carp and tilapia by the Chemiluminescecent Immunoassay (CLIA) analytical methods.

Collection blood and analysis of the three species of fish blood hormones value before and after anesthetization

Before anesthetization

Pick the three domesticated species fish in the barrel at random, and then by collecting blood of injection apparatus〔needles（ $18G \times 1\frac{1}{2}"$ ） injection and syringe of 10ml〕. Collected small artery blood from the caudal vein of fish body. Treatments of blood were not dealt with the two anesthetics. Poured into the adopt blood vessel that

includes heparin injection solution and shaked artificially. Put the blood into a tube which centrifuge to separate. About 20 minutes later (but the Common carp blood must spend 100 minutes), collected the upper clear liquid to pack into the adopt blood vessel gor analysis. Analysis of the data with nonparametric statistical method.

After anesthetization

Pick some fish, anesthetized with the two anesthetic and pick fishes randomly, collected blood by heparin sodium injection apparatus〔needles(18G ×1 1/2 ")injection and syringe of 10 ml〕, Collected the small artery blood from the caudal vein of fish body. After the blood which had been divided into the two anesthetics were dealt, poured into the adopted blood vessel that includes heparin sodium injection. Shaked artificially then put the blood into a tube and centrifuge to separate. About 20 minutes later (but the Common carp blood must spend 100 minutes), collected the upper clear liquid to pack into the adopted blood vessel for analysis. Analysis of the data with nonparametric statistical method.

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 21") injection and syringe of 10ml]. 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.

CONCLUSION

In this study, the values of triiodothyronine and thyroxine were significantly different before and after anesthetization in tilapia with local anesthetics. other hormones, luteinizing hormone and follicle stimulating hormone, which do not change in tilapia before and after anesthetization with local anesthetics.

This work emphasizes the stress of anaesthesia. Some hormones of tilapia are not the stress hormones, for instance, luteinizing hormone and follicle stimulating hormone. Some hormones are stress hormones, for instance, triiodothyronine (T3) and thyroxine (T4). T3 and T4 values are significantly different before and after anaesthetizing in

the analyses of fish blood, and the changed range was also to be different because of different class local anesthetics.

The inspection of examining fish blood with medical instrument is the innovation, and usability, and may establish a connecting channel of both fishery medicine and human medicine. It is very practical and innovation adding for fishery science and human medicine.

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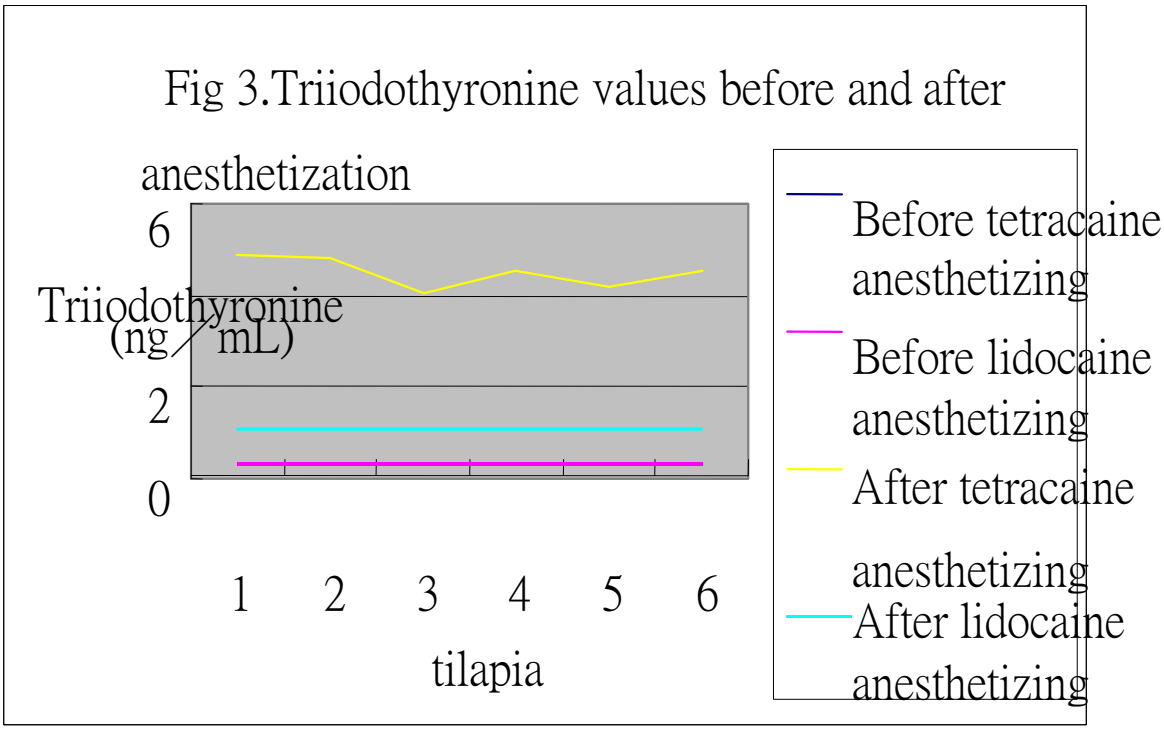

Figure 1. Triiodothyroninevalues before and after anesthetization

TABLE 1 The luteinging hormone values before and after anesthetization in tilapia

Treatments	lidocaine		tetracaine	
	Before	After	Before	After
	0.31	0.31	0.33	0.33
	0.34	0.34	0.25	0.25
Results	0.31	0.31	0.42	0.42
(mIU／mL)	0.38	0.38	0.31	0.31
	0.33	0.33	0.33	0.33
	0.34	0.34	0.32	0.32

TABLE 2 The follicle stimulating hormone values before and after anesthetization in tilapia

Treatments	lidocaine		tetracaine	
	Before	After	Before	After
	0.32	0.32	0.32	0.32
	0.31	0.31	0.35	0.35
Results	0.39	0.39	0.22	0.22
(mIU／mL)	0.33	0.33	0.41	0.41
	0.31	0.31	0.47	0.47
	0.33	0.33	0.32	0.32

TABLE 3. The triiodothyronine values before and after anesthetization in tilapia

Treatments	lidocaine		tetracaine	
	Before	After	Before	After
	0.09	5.56*	0.52	5.17*
	1.54	5.98*	0.54	5.14*
Results	1.34	5.58*	0.54	5.09*
(ng／mL)	1.70	6.12*	0.51	5.71*
	1.83	7.25*	0.57	5.18*
	1.39	6.05*	0.54	5.32*

* The asterisk indicate significant differences.

TABLE 4 The thyroxine values before and after anesthetization in tilapia

Treatments	lidocaine		tetracaine	
	Before	After	Before	After
	1.12	21.73*	1.22	7.56*
	1.14	21.33*	1.38	7.68*
Results	1.07	20.58*	1.34	7.64*
(ug／dL)	1.30	21.75*	1.37	7.82*
	2.74	23.83*	1.25	7.62*
	1.75	22.55*	1.38	7.59*

* The asterisk indicate significant differences.

Table 5. Blood property test of lidocaine treated *Oreochromis mossambicus*

Items	Before treatment	After treatment
WBC	167.2 ± 2.43 10^3/ul	167.8 ± 3.57 10^3/ul
RBC	1.81 ± 0.26 10^6/ul	2.24 ± 0.24 10^6/ul
HGB	10.6 ± 0.45 g/dl	12.8 ± 0.72 g/dl
HCT	28.35 ± 3.30 %	36.25 ± 2.6 %
MCV	60.56 ± 2.20 fL	61.82 ± 1.95 fL
MCH	37.17 ± 2.01 pg	37.26 ± 2.50 pg
MCHC	36.87 ± 0.75 g/dL	36.45 ± 0.80 g/dL
PLT	96.67 ± 5.50 10^3/ul	19.87 ± 1.50 10^3/ul
RDW-SD	44.60 ± 4.7 fL	46.65 ± 4.05 fL
RDW-CV	15.37 ± 1.06 %	9.12 ± 1.05 %
MPV	10.75 ± 0.05 fL	11.27 ± 0.10 fL
PCT	0.04 ± 0.02 %	0.06 ± 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〉

Table 6. Blood property test of tetracaine treated *Oreochromis mossambicus*

Items	Before treatment	After treatment
WBC	166.35 ± 2.63 10^3/ul	167.12 ± 2.87 10^3/ul
RBC	1.61 ± 0.16 10^6/ul	2.01 ± 0.44 10^6/ul
HGB	12.6 ± 0.25 g/dl	15.2 ± 0.32 g/dl
HCT	29.15 ± 2.30 %	37.25 ± 2.49 %
MCV	178.56 ± 2.20 fL	182.82 ± 1.95 fL
MCH	71.37 ± 3.61 pg	72.86 ± 2.62 pg
MCHC	43.87 ± 0.25 g/dL	43.45 ± 0.60 g/dL
PLT	72.37 ± 3.56 10^3/ul	13.87 ± 3.50 10^3/ul
RDW-SD	44.60 ± 4.7 fL	46.65 ± 4.05 fL
RDW-CV	18.32 ± 1.12 %	8.62 ± 1.48 %
MPV	11.75 ± 0.08 fL	11.67 ± 0.11 fL
PCT	0.04 ± 0.01 %	0.05 ± 0.03 %

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〉