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Hematological effects of etomidate and tricaine in common carp

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Abstract: Hematological effects of etomidate and tricaine at concentrations causing general anesthesia or sedation of carp juveniles were compared. Recovery of body balance took similar time in the case of both anesthetics, but fish treated with etomidate were depressed for several hours, while those subjected to tricaine immediately regained normal behavior. Immediately after exposure anesthetics caused minor hematological changes; the only statistically significant effects (decrease of hematocrit and plasma glucose) occurred after sedation with etomidate. One week after exposure a significant increase in mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration occurred in all groups of fish compared to the previous values, with slight increase in hemoglobin and decrease in red blood cell count. The mean corpuscular volume values tended to increase in all groups except for the control. Oxidative metabolic activity of phagocytes was significantly higher in both groups treated with etomidate compared to the control and tricaine groups. The results revealed that etomidate was less aversive and better prevented a delayed immunosuppressive effect of stress caused by handling and bleeding, while very quick complete recovery was the benefit of tricaine. These findings suggest that etomidate is better when long-term stress prevention is required, while tricaine is more useful for short-term immobilization of fish when rapid recovery of normal behavior is needed.

Key words: Anesthesia, blood, erythrocytes, leukocytes, fish

1. Introduction

Anesthetics are used in aquaculture to improve fish welfare and to prevent stress during harvest, handling, transport, and other procedures (1,2). They are also used in research for sedation, general anesthesia, or euthanasia of fish (3).

Tricaine (MS-222) is likely the most common anesthetic agent used for fish but others, such as etomidate, are also commonly used (4). Both chemicals are considered safe for fish (3,5). Etomidate is an imidazole-based nonbarbiturate hypnotic agent that potentiates the activity of gamma-aminobutyric acid receptors (6) and inhibits neurotransmitter release (7), while tricaine (ethyl 3-aminobenzoate methanesulfonic acid) is known to block voltage-gated sodium channels (8). According to Kristan et al. (9), time of anesthesia induction and recovery is much shorter for tricaine compared to etomidate. Etomidate, despite its effect on the nervous system, shows an inhibitory effect on steroidogenesis (10,11), which results in a decrease in cortisol level and thus suppressed stress response (12). Tricaine was reported to cause stress reactions by increasing cortisol release in fish (13). A comparison of plasma cortisol responses to various anesthetics showed that metomidate caused no change in plasma cortisol concentration, while tricaine induced an increase (14). On the other hand, Hill and Forster (15) found that metomidate caused deeper hypoxemia than tricaine, which implies possible compensatory hematological changes. The results obtained by Readman et al. (4) indicated that tricaine induced aversive behavioral responses in fish, while no such reaction was observed in fish treated with etomidate.

Taking into consideration different modes of action and different effects of etomidate and tricaine on stress responses in fish, we can also expect different hematological effects of these anesthetics and thus their possible interference with the effects of experimental factors. The data on hematological effects of both anesthetics are incomplete and often contradictory. According to Lepic et al. (16), MS-222 and etomidate produced no distinct hematological effects in *Vimba vimba*, while Kristan et al. (17) reported that they caused leukopenia in *Sander lucioperca*. Limsuwan et al. (12) observed increases in hematocrit, hemoglobin concentration, and erythrocyte count in *Ictalurus punctatus* and Witeska et al. (18) found an increase in mean cell hemoglobin level and frequency of abnormal erythrocytes in *Cyprinus carpio* treated with etomidate, while Kazun and Siwicki (5) reported no change in the values of hematological parameters in

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eight other species of fish. According to Gressler et al. (14), MS-222 induced hematocrit increase in *Rhamdia quelen*, while Bishkoul et al. (19) observed reduction in hemoglobin concentration and hematocrit accompanied by erythropenia and leukopenia in *Acipenser ruthenus* subjected to this anesthetic. Limited data are available on the effects of etomidate and tricaine on immune responses in fish. Cubero and Molinero (20) reported transient neutropenia and basophilia in *Sparus aurata* subjected to MS-222 anesthesia. Ortuno et al. (21) observed no depression of various phagocyte functions in the same species such as respiratory burst and phagocytic ability, while according to Gholipour Kanani et al. (22) MS-222 reduced respiratory burst of phagocytes. Our previous study (18) revealed that deep anesthesia of carp with etomidate resulted in neutrophilia, monocytosis, and thrombocytosis.

The aim of the present study was to compare hematological effects of two anesthetics, etomidate and tricaine, used at two concentrations causing deep general anesthesia or deep sedation.

2. Materials and methods

Common carp juveniles of 35.5 ± 8.3 g (about 5 months old), harvested in October 2014 from the rearing pond of the Inland Fisheries Institute in Żabieniec, were brought to the laboratory of the Department of Animal Physiology, Siedlce University of Natural Sciences and Humanities, in plastic bags with water and pure oxygen. Ichthyopathological examination revealed that the fish were clinically healthy. The fish were kept for 3 weeks in a flow-through aerated tank. Water quality parameters were measured twice a week (water temperature 15.6–17.2 °C, dissolved oxygen concentration 7.1–8.9 mg/L, ammonia 0.0–1.0 mg/L, nitrite 0.0–0.1 mg/L, and water pH 7.5–8.1). During this time the fish were fed every morning to satiation with Aller-Aqua Classic carp feed (4.5 mm). No mortalities or disease symptoms were observed during the acclimation period.

One week before the experiment 60 fish were randomly transferred to 5 glass aquaria of 100 L in volume with 12 fish in each. During this period and during the experiment water was constantly aerated and changed daily after feeding (2/3 of water was siphoned out without disturbing fish and replaced with fresh water so as not to disturb fish). Water quality parameters in aquaria were measured daily before water replacement (water temperature 15.1–17.3 °C, dissolved oxygen concentration 8.8–9.7 mg/L, ammonia 0.0–1.0 mg/L, nitrite 0.0–0.1 mg/L, and water pH 8.0–8.5). The fish were fed every morning with Aller-Aqua Classic at the rate of 1% of fish body mass per day. On the days of blood sampling the fish were fed after completion of the procedure. No symptoms of disease or mortalities were observed during the experiment.

During the experiment the fish from two tanks (Eto1, Eto2) were subjected to 30 min of deep anesthesia or deep sedation with etomidate (16 or 8 mg/L, respectively), while the fish from Tri1 and Tri2 tanks were subjected to tricaine methanesulfonate (75 or 50 mg/L) solutions. Concentrations of anesthetics were established as described by Witeska et al. (18). Before this procedure the water level in the aquaria was lowered to 50 L. Solutions of the anesthetics were added directly to the water and after 30 min the fish were gently transferred with a net to neighboring identical tanks with fresh aerated water to recover. The control group was subjected to the same manipulations as experimental fish (reduction of water level and transfer to a neighboring tank with fresh water). The 2% etomidate solution was stabilized with a neutral organic compound (Propiscin, Inland Fisheries Institute, Poland), and tricaine methanesulfonate (ethyl 3-aminobenzoate methanesulfonate, MS-222, Sigma Aldrich, China) was used. Water temperature during exposure to anesthetics was 17 °C and dissolved oxygen concentration ranged from 8.6 to 9.3 mg/L. After 1 h of recovery blood was sampled from all fish by heart puncture with heparinized needles into heparinized Eppendorf tubes (about 150 µL from each fish, n = 12). Hematocrit (Ht), hemoglobin concentration (Hb), erythrocyte count (RBC), mean cell volume (MCV), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), and leukocyte count (WBC) were evaluated according to Svobodova et al. (23). Briefly, Ht was measured after centrifugation of blood in glass capillaries (2 replicates for each fish) for 3 min at 12,000 rpm. Hb was measured using a spectrophotometric cyanmethemoglobin method at 540 nm. RBC and WBC were calculated with a microscope cytometric method using a Bürker hemocytometer in blood diluted with Hayem solution (1:100). Spontaneous oxidative activity of phagocytes was measured according to Studnicka et al. (24) using the nitrotetrazolium blue (NBT) reduction method. Concentration of the obtained product (formazan) was measured using a spectrophotometer at 546 nm wavelength. Blood glucose concentrations were measured with an Accu-Check (Roche, Switzerland) glucometer. Blood smears were also made and stained with May–Grünwald and Giemsa solutions for evaluation of red and white blood cell populations. In each smear, 300 erythrocytes and 100 leukocytes were inspected. Percentages of abnormal erythrocytes (showing nuclear or cellular anomalies) and erythroblasts were calculated as well as percentages of various types of leukocytes: lymphocytes, neutrophils, monocytes, and other. Since the percentages of neutrophils and monocytes were very low and did not show significant differences among groups or in time, they were summed and showed in the tables as phagocytes. The numbers of thrombocytes per 100

leukocytes were counted in the smears and their count (PLT) was calculated based on WBC, according to the formula PLT = (a × WBC) / 100.

The obtained results (shown in tables as arithmetic mean ± SD) were subjected to statistical analysis using Statistica 10 (StatSoft, USA) software. The results of Shapiro–Wilk tests revealed nonnormal distribution of most variables; therefore, the nonparametric Kruskal–Wallis test (assuming P £ 0.05) was used to evaluate the significance of differences among the groups.

The study was approved by the III Local Ethical Committee at the Warsaw University of Life Sciences.

3. Results

Behavior of fish was observed during induction of anesthesia and recovery. Fish treated with both concentrations of etomidate did not show any agitation at the beginning of exposure. At 16 mg/L etomidate (Eto1), fish lost body equilibrium after 5 min, and after 15 min reaction to stimuli (gentle striking with a glass rod) ceased. Decrease of ventilation frequency was observed after 20 min. Recovery of body equilibrium took about 30 min but the fish remained depressed for over 12 h and stayed almost motionless at the bottom of the tank. In fish treated with 8 mg/L etomidate (Eto2) body balance was disturbed; reaction to stimuli was suppressed but ventilation was normal. Recovery of body balance took about 10 min but the fish remained slowed down for another several hours. In the case of tricaine, at the beginning of exposure all fish showed a short period of increased locomotor activity and gasping of air. At 75 mg/L fish lost body equilibrium within 5 min, and they became unreactive after 10 min, simultaneously with the decrease of ventilation frequency. After 15 min very weak ventilator movements were observed. Complete recovery of body equilibrium, ventilation, and behavior took place after 10 min. At 50 mg/L tricaine fish lost body balance and became almost unreactive after 20 min; at the end of exposure ventilation was slightly depressed. Complete recovery was observed within 5–10 min.

In 1 h after exposure to anesthetics only minor hematological alterations occurred (Table 1). Fish sedated with 8 mg/L etomidate (Eto2) showed significantly reduced Ht and blood glucose level and insignificantly lower Hb, RBC, WBC, PLT, and NBT values compared to the remaining groups.

More pronounced differences were observed 1 week from exposure (Table 2). In all groups of fish, including the control group, a significant increase in MCH and MCHC values occurred, accompanied by an increase in Hb and decrease in RBC (insignificant in most groups). Erythrocyte volume (MCV) also tended to increase in all groups except for the control, where it remained unchanged. The values of oxidative metabolic activity of phagocytes (NBT) were significantly higher in both groups exposed to etomidate compared to the control and groups treated with tricaine. Blood glucose significantly increased in Eto1 and decreased in Tri2. No alterations

Table 1. Immediate (1 h after treatment) effects of deep anesthesia and deep sedation with etomidate or tricaine in common carp (arithmetic mean ± SD, values with different lowercase letter superscripts differ among the groups, Kruskal–Wallis test, P ≤ 0.05).

	Control	Eto1	Eto2	Tri1	Tri2
Ht [%]	25.5 ± 2.1 ^{a}	24.4 ± 2.9 ^{a}	20.8 ± 3.3 ^{b}	22.5 ± 3.0 ^{a}	24.9 ± 3.4 ^{a}
Hb [g/L]	38.2 ± 13.4 ^{a}	43.9 ± 7.7 ^{a}	37.6 ± 4.7 ^{a}	43.2 ± 19.4 ^{a}	45.3 ± 18.8 ^{a}
RBC [10^6/μL]	1.46 ± 0.17 ^{a}	1.44 ± 0.20 ^{a}	1.21 ± 0.20 ^{a}	1.37 ± 0.30 ^{a}	1.69 ± 0.51 ^{a}
MCH [pg]	26.6 ± 10.2 ^{a}	31.0 ± 6.1 ^{a}	31.3 ± 3.8 ^{a}	31.6 ± 11.8 ^{a}	28.6 ± 14.8 ^{a}
MCHC [g/L]	150 ± 478 ^{a}	181 ± 32 ^{a}	184 ± 28 ^{a}	189 ± 71 ^{a}	184 ± 77 ^{a}
MCV [fL]	176 ± 19 ^{a}	172 ± 22 ^{a}	172 ± 21 ^{a}	170 ± 41 ^{a}	156 ± 44 ^{a}
Erythroblasts [%]	1.2 ± 0.5 ^{a}	0.3 ± 0.3 ^{a}	0.6 ± 0.7 ^{a}	1.0 ± 0.7 ^{a}	0.5 ± 0.5 ^{a}
WBC [10^3/μL]	91.3 ± 31.9 ^{a}	80.9 ± 21.9 ^{a}	73.3 ± 27.0 ^{a}	89.4 ± 38.1 ^{a}	99.4 ± 30.2 ^{a}
Lymphocytes [%]	99.1 ± 0.8 ^{a}	98.5 ± 1.0 ^{a}	96.5 ± 1.5 ^{a}	98.0 ± 1.8 ^{a}	95.2 ± 4.0 ^{a}
Phagocytes [%]	0.9 ± 0.8 ^{a}	1.3 ± 0.8 ^{a}	3.5 ± 1.5 ^{a}	1.9 ± 1.8 ^{a}	4.5 ± 3.7 ^{a}
PLT [10^3/μL]	11.0 ± 5.8 ^{a}	7.7 ± 5.9 ^{a}	5.8 ± 4.7 ^{a}	6.6 ± 5.3 ^{a}	9.7 ± 4.5 ^{a}
NBT [g/L]	0.83 ± 0.21 ^{a}	0.80 ± 0.31 ^{a}	0.74 ± 0.20 ^{a}	1.02 ± 0.31 ^{a}	0.87 ± 0.18 ^{a}
Glucose [mg/dL]	65.0 ± 11.5 ^{a}	49.6 ± 6.4 ^{ab}	43.9 ± 9.0 ^{bA}	81.7 ± 8.6 ^{ac}	80.9 ± 15.2 ^{ac}

Table 2. Delayed (1 week after treatment) effects of deep anesthesia and deep sedation with etomidate or tricaine in common carp (arithmetic mean ± SD, values with different lowercase letter superscripts differ among the groups, asterisks indicate values significantly different from those obtained in the same groups at 1 h after treatment, Kruskal–Wallis test, $P \leq 0.05$).

	Control	Eto1	Eto2	Tri1	Tri2
Ht [%]	21.2 ± 4.3 ^{a}	23.3 ± 2.7 ^{a}	20.8 ± 3.9 ^{a}	23.1 ± 4.6 ^{a}	19.3 ± 2.1 ^{a*}
Hb [g/L]	59.6 ± 10.9 ^{a}	64.6 ± 11.5 ^{a}	58.8 ± 10.3 ^{a*}	65.5 ± 11.9 ^{a*}	59.0 ± 6.4 ^{a}
RBC [10^6/μL]	1.21 ± 0.24^{ab}	0.97 ± 0.26 ^{a*}	0.89 ± 0.22 ^{a}	1.19 ± 0.19 ^{a}	1.16 ± 0.19 ^{b*}
MCH [pg]	49.9 ± 6.8 ^{ab*}	72.1 ± 24.3 ^{a*}	68.7 ± 15.5 ^{a*}	55.3 ± 5.5 ^{a*}	52.0 ± 8.5 ^{b*}
MCHC [g/L]	284 ± 30 ^{a*}	277 ± 35 ^{a}	286 ± 33 ^{a*}	287 ± 38 ^{a*}	310 ± 53 ^{a*}
MCV [fL]	176 ± 24 ^{ab}	260 ± 89 ^{a}	239 ± 41 ^{a*}	195 ± 28 ^{a}	169 ± 21 ^{b}
Erythroblasts [%]	1.4 ± 1.3 ^{a}	1.1 ± 0.8 ^{a}	0.5 ± 0.7 ^{a}	1.1 ± 1.5 ^{a}	1.3 ± 2.0 ^{a}
WBC [10^3/μL]	76.4 ± 23.2 ^{ab}	44.7 ± 13.5 ^{a*}	72.4 ± 23.2 ^{ab}	71.0 ± 26.1 ^{ab}	98.8 ± 19.4 ^{b}
Lymphocytes [%]	95.1 ± 3.5 ^{a}	96.4 ± 2.7 ^{a}	95.4 ± 2.9 ^{a}	98.1 ± 1.6 ^{a}	95.6 ± 1.9 ^{a}
Phagocytes [%]	4.4 ± 2.9 ^{a}	3.3 ± 2.4 ^{a}	4.2 ± 3.2 ^{a}	1.9 ± 1.6 ^{a}	3.9 ± 1.8 ^{a}
PLT [10^3/μL]	9.3 ± 5.8 ^{a}	5.4 ± 4.9 ^{a}	5.0 ± 4.9 ^{a}	4.2 ± 3.9 ^{a}	15.8 ± 11.6 ^{a}
NBT [g/L]	0.59 ± 0.09 ^{a}	1.00 ± 0.21 ^{b}	0.92 ± 0.21 ^{b}	0.67 ± 0.19 ^{a}	0.75 ± 0.22 ^{a}
Glucose [mg/dL]	52.4 ± 7.6 ^{a}	64.3 ± 16.2 ^{a*}	49.8 ± 8.9 ^{a}	61.8 ± 12.4 ^{a}	74.0 ± 15.9 ^{a*}

in erythroblast frequency were observed and the level of abnormal erythrocytes was very low (0.2%–0.7%). No significant differences in differential leukocyte count or thrombocyte level (PLT) took place.

4. Discussion

The observed differences in fish behavior showed that etomidate, contrary to tricaine, did not cause agitation, but complete recovery from anesthesia required much more time. Similar results were obtained by other authors. Readman et al. (4) reported that tricaine was aversive in *Brachydanio rerio*, which was indicated by avoidance, while etomidate did not induce such a reaction. Longer recovery of fish from etomidate anesthesia compared to that obtained with tricaine was reported by Kristan et al. (9). Nordgreen et al. (25) observed that tricaine had no effect on fish swimming behavior, which returned to a normal pattern immediately after recovery from anesthesia.

Hematological results obtained 1 h after treatment revealed that both anesthetics affected the values of blood parameters very little in common carp juveniles. In our previous experiment (18) we reported increases in MCH, erythroblastosis, and frequency of abnormal erythrocytes after deep anesthesia with etomidate, which did not take place in the present study. This is difficult to explain and might have resulted from different sensitivity of both groups of fish to anesthetics: those used in the present experiment required higher concentrations of Propiscin to induce sedation and anesthesia compared to the fish used by Witeska et al. (18). Lack of significant hematological effects of the same anesthetics in *Vimba vimba* was also observed by Lepic et al. (16). The most extensive study concerning hematological effects of etomidate on fish was performed by Kazun and Siwicki (5). They reported no alterations in red blood indices of RBC, Ht, Hb, MCV, MCH, and MCHC in *Cyprinus carpio*, *Ctenopharyngodon idella*, *Clarias gariepinus*, *Lota lota*, *Oncorhynchus mykiss*, *Hucho hucho*, *Salmo trutta* m. *fario*, and *Thymallus thymallus*. However, increases in RBC, Ht, and Hb were observed in *Ictalurus punctatus* by Limsuwan et al. (12), and increases in MCHC and leukopenia in *Sander lucioperca* were reported by Kristan et al. (17). Stockman et al. (26) observed no changes in hematocrit level in *Cyprinus carpio* following treatment with various concentrations of MS-222 (50–190 mg/L). However, various authors reported hematological changes in fish following MS-222 anesthesia. Matsche (27) found a transient erythrocyte swelling and a delayed (24 h after exposure) increase in RBC, leukopenia, and neutrophilia in *Acipenser oxyrinchus*. Bishkoul et al. (19) reported erythropenia and leukopenia in *Acipenser ruthenus*. Significant increase in Ht and Hb was observed in *Sparus aurata* by Molinero and Gonzalez (28). Increase in Ht accompanied by other changes indicating stress, such as cortisol release, plasma ion loss, and lipid peroxidation, was reported by Gressler et al. (14) for *Rhamdia quelen*.

The changes in red blood cell parameters observed 1 week after exposure in all groups of fish included significant increases in MCH and MCHC (accompanied by an increase in Hb and decrease in RBC, although these changes were insignificant in most groups) compared to the values obtained 1 h after treatment. Occurrence of the changes also in the control group indicates that they did not result from anesthesia or sedation but were a compensatory reaction to handling and bleeding. These changes suggest an increase in hemoglobin level in circulating cells since no increased release of erythroblasts took place. Increase of hemoglobin concentration in fish erythrocytes with age and its synthesis in circulating cells was confirmed by Lund et al. (29). The values of oxidative metabolic activity of phagocytes reported 1 week after exposure suggest that etomidate, contrary to tricaine, showed a beneficial effect on the nonspecific immune potential of fish. Inhibition of the immunosuppressive effect of handling stress (lymphopenia, neutrophilia, and reduction of phagocyte oxidative metabolic activity) in carp juveniles sedated with etomidate was also observed in our previous study (18). Palic et al. (30) reported that metomidate prevented stress-induced decrease of neutrophil function in *Pimephales promelas*, while MS-222 did not. Ortuno et al. (21) found that tricaine did not suppress respiratory burst and other phagocyte functions in *Sparus aurata*. On the other hand, Gholipour Kanani et al. (22) reported that spontaneous respiratory burst of *Oncorhynchus mykiss* phagocytes was strongly depressed 24 h after MS-222 anesthesia.

The obtained results revealed that both anesthetics caused few physiological changes but their advantages are different: etomidate seems less aversive and may better prevent delayed immunosuppressive effects of stress, while very quick complete recovery is the benefit of tricaine. Therefore, etomidate can be recommended particularly when sedation and long-term stress prevention are required, while tricaine may be more useful for short-term immobilization of fish when rapid recovery of normal swimming behavior is required.

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