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Influence of 1-phenoxy-2-propanol on blood profile of common carp

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Abstract

1-phenoxy-2-propanol is a common and effective anaesthetic for aquatic organisms such as bivalves and pulmonates. However, there are no data regarding its influence on fish organisms. In the present study the anaesthetic efficacy of 1-phenoxy-2-propanol and its influence on biochemical and haematological blood indices of juvenile common carp were studied. For blood profile test, fish were divided into four groups (n = 10). The haematological and blood biochemical profiles of common carp were evaluated 10 min and 24 h after anaesthesia with 1-phenoxy-2-propanol (400 $mg \cdot dm^{-3}$) and compared to non-anaesthetized control groups. Significant changes ($P < 0.05$) in red blood cell indices and in white blood cell count were found as well. Increased concentrations of glucose, ammonia and inorganic phosphates indicate that stress reaction occurred. No changes in total protein, globulin, triacylglycerols, alkaline phosphatase, aspartate aminotransferase and calcium were found. Although exposure to 1-phenoxy-2-propanol caused a moderate, temporary stress response in examined fish, we can state that 1-phenoxy-2-propanol can be used as an effective anaesthetic for common carp.

Propylene phenoxytol, anaesthesia, biochemistry, haematology, Cyprinus carpio

The beneficial influence of anaesthetics and analgesics has been known for centuries, however, they have been used in fish anaesthesia for a particularly short period of time. First reports about anaesthetizing fish refer to anaesthesia of salmon and steelhead trout with carbon dioxide (Fish 1943). From this time several different chemicals such as MS-222 (Randall 1962), 2-phenoxyethanol (Sehedev et al. 1963), quinaldine (Schoettger and Steucke 1970), benzocaine (Oswald 1978), etomidate (Amend 1982), metomidate (Stoskopf and Arnold 1985), clove oil (Hisaka 1985) and propofol (Fleming et al. 2003) were tested as anaesthetics for fish. However, because of marked anatomical, physiological and behavioral variations, there are still no anaesthetics which can be used for all fish species. So it is very important to seek new, potentially better anaesthetics.

1-phenoxy-2-propanol (PP) is the analogue of 2-phenoxyethanol (2PE). It is a glycol ether forming a clear, colourless liquid at room temperature. Although relatively hydrophobic, it can be dissolved in water, however, to a lower extent compared to 2PE. The solubility of 2PE and PP are 27 and 11 $g \cdot dm^{-3}$, respectively. 1-phenoxy-2-propanol is widely used in the study of invertebrates. It has been proved that PP can be used to relax or anaesthetize pulmonates (Runham et al. 1965), bivalves (Owen 1955; Norton et al. 1996; Norton et al. 2000) and nudibranchs (Holman et al. 2002; Redondo and Murray 2005; Wyeth and Willows 2006b). It was also used as an anaesthetic for rats (Saghir et al. 2003). However, although it was found to be effective, its anaesthetic action has not been thoroughly investigated. No data addressing its anaesthetic efficacy or toxicity to fish are available.

The aim of this study was to assess the anaesthetic potency of PP and its influence on biochemical and haematological blood indices of common carp.

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Materials and Methods

Fish

The experiment was carried on young carp (n = 40) of 107 ± 20.9 g of weight and 190 ± 11.9 cm in total length. Fish were supplied by local Fish Farm Ostróda, Poland. Fish were acclimated for two weeks before experiment in 0.3 m^3 tanks and fed commercial pellet feed. The water temperature during acclimation and the experiment was 19.5 ± 0.5 °C.

Anaesthetic

For biochemical and haematological tests, 1-phenoxy-2-propanol (PP) supplied by Sigma Aldrich (USA) was used. Before the experiment, a working solution of PP in ethyl alcohol (50 $g \cdot dm^{-3}$) was prepared.

Exposure

The procedure proposed by Velíšek and Svobodová (2004 a,b) and Velíšek et al. (2006) was applied. Fish were randomly caught from the tank and individually subjected to one of the following procedures. Control fish (CF) (n = 10) were blood sampled immediately (within less than 2 min, without anaesthesia) after the catch. Treatment 1 fish (T1) (n = 10) were exposed to water solution of 400 $mg \cdot dm^{-3}$ of PP for 10 min, and blood was sampled immediately after exposure; treatment 2 fish (T2) (n = 10) were exposed to PP as above and then moved to the tank (0.3 m^3 of volume) filled with anaesthetic-free water for recovery. Blood was sampled 24 h after exposure. Stress-exposed control fish (SEC) (n = 10) were caught from the rearing tank and placed in an exposure box filled with anaesthetic-free water for 10 min and then moved to a 0.3 m^3 tank for recovery. Blood was sampled after 24 h. Both T2 and SEC fish were blood-sampled without anaesthesia. The exposure was done in a 12 dm^3 polypropylene box. The PP solution was aerated mechanically. Bath temperature was the same as in the rearing tank water.

Blood analysis

Blood was sampled from caudal vessels by a syringe covered with heparin lithium salt. Approximately 0.8 ml of the blood was centrifuged in StatSpin centrifuge at 12,000 *g* for 30 s. Blood plasma was collected for biochemical analysis and immediately frozen at -20 °C. The rest of collected blood was used for blood smear preparation (2 smears per each fish) and determination of haematological indices. After blood sampling, fish were placed in excessive propofol solution (20 $mg \cdot dm^{-3}$). Following the arrest of an opercular movement, fish brain was destroyed with sharp scissors and the length (LC) and weight measurements were taken.

Haematological indices were determined according to standard methods given in Unified Methods for Haematological Examination of Fish (Svobodova et al. 1986) and covered: erythrocyte count (RBC), haemoglobin concentration (Hb), haematocrit (PCV), mean erythrocyte volume (MCV), mean corpuscular haemoglobin concentration (MCHC), mean corpuscular haemoglobin (MCH), leukocyte count (WBC) and the differential leukocyte count (leukogram).

Plasma samples were analyzed with Catalyst Dx Chemistry Analyzer (Idexx Lab., USA). Analysis of biochemical indices included: inorganic phosphates (PHOS), calcium (Ca), total protein (TP), albumin (ALB) and globulin (GLOB), ammonia (NH_3), triacylglycerols (TRIG), glucose (GLU), aspartate aminotransferase (AST), and alkaline phosphatase (ALKP). Each plasma sample was thawed only once at room temperature and all the above listed indices were determined at one run of the chemistry analyser.

Statistical analysis

Statistica StatSoft 12.0 software was used for statistical analysis. To test normality and variance homogeneity, Shapiro-Wilk's and Leven's tests, respectively, were used. Differences between groups were tested with univariate ANOVA and nonparametric Kruskall-Wallis tests. The differences were considered as significant at $P \leq 0.05$.

Results

No mortalities were observed 24 h following anaesthesia. The exposure of fish to PP resulted in significant ($P < 0.05$) changes in red blood indices (Table 1). The RBC values were significantly higher in both anaesthetized groups as well as in SEC fish compared to CF. Significantly higher values of PCV were determined in T1 fish compared to CF. However, they returned to the initial values over the next 24 h. The Hb values were significantly lower 24 h after exposure in T2 and SEC fish. The MCV, MCHC, and MCH values were significantly decreased in both anaesthetized groups as well as in the SEC group.

Table 1. Changes in haematological blood indices of common carp during PP anaesthesia.

Indices	Treatment			
	CF	T1	SEC	T2
RBC [T·dm^{-3}]	0.97 ± 0.26^{a}	1.53 ± 0.22^{b}	1.27 ± 0.32	1.55 ± 0.3^{b}
PVC	0.29 ± 0.04^{a}	0.37 ± 0.02^{b}	0.32 ± 0.02^{a}	0.3 ± 0.02^{a}
Hb [g·dm^{-3}]	83.54 ± 6.74^{a}	85.98 ± 2.80^{a}	61.92 ± 5.64^{b}	63.98 ± 8.7^{b}
MCV [fl]	327.6 ± 77.8^{a}	243.53 ± 48.86^{b}	263.88 ± 74.19^{b}	201.71 ± 50.29^{b}
MCHC [g·dm^{-3}]	277.7 ± 20.7^{a}	233.91 ± 14.60^{b}	196.94 ± 18.03^{b}	211.95 ± 18.32^{b}
MCH [pg]	91.6 ± 25.8^{a}	57.54 ± 10.05^{b}	52.69 ± 19.23^{b}	43.12 ± 13.89^{b}
WBC [g·dm^{-3}]	78.25 ± 9.13^{a}	52.75 ± 7.94^{b}	44.00 ± 5.91^{b}	56.75 ± 16.87^{b}

Groups with different alphabetic superscripts differ significantly at $P < 0.05$ (ANOVA)
RBC – red blood cell, PCV – haematocrit, Hb – haemoglobin, MCV – mean corpuscular volume, MCHC – mean corpuscular haemoglobin concentration, MCH– mean corpuscular haemoglobin, WBC – white blood cell, CF – control fish, SEC – stress exposed control fish, T1 – treatment 1 fish, T2 – treatment 2 fish, PP – 1-phenoxy--2-propanol

A significant decrease of WBC was noticed in both anaesthetized groups as well as in SEC fish compared to CF. It was mainly due to the decrease of the lymphocyte count (Table 2).

Table 2. Changes in differential leukocyte counts in common carp during PP anaesthesia.

Indices	Treatment			
	CF	T1	SEC	T2
Lymphocytes [G·dm^{-3}]	33.49 ± 7.16^{a}	20.31 ± 6.59^{b}	17.38 ± 6.22^{b}	22.08 ± 7.59^{b}
Segmented granulocytes [G·dm^{-3}]	1.29 ± 0.36^{a}	1.13 ± 0.59^{a}	1.08 ± 0.52^{a}	1.45 ± 0.42^{a}
Banded granulocytes [G·dm^{-3}]	1.30 ± 0.75^{a}	1.13 ± 1.01^{a}	1.67 ± 0.89^{a}	1.33 ± 0.92^{a}
Developmental phases – myeloid sequence [G·dm^{-3}]	2.20 ± 1.11^{a}	2.18 ± 1.50^{a}	1.96 ± 1.34^{a}	2.24 ± 1.79^{a}

Groups with different alphabetic superscripts differ significantly at $P < 0.05$ (ANOVA)
CF – control fish, SEC – stress exposed control fish, T1 – treatment 1 fish, T2 – treatment 2 fish, PP – 1-phenoxy-2-propanol

After a 10 min exposure to PP, no changes in total protein blood plasma and globulin concentration were found. However, PP anaesthesia caused a significant increase of albumin concentration 24 h following exposure in T1, T2, and SEC fish.

The significant increase of glucose concentration was observed in T1 and T2 fish. Also SEC fish showed elevated blood plasma glucose concentration (Fig. 1a). Triacylglycerol concentrations were not affected and ranged between 1.66 and 1.99 mmol·dm^{-3}.

A significant increase of inorganic phosphate concentration was observed in T1 fish, however, it was restored within 24 h after exposure (Fig. 1b). Phosphate was also increased in SEC fish, however, it was not significantly different compared to control.

Anaesthesia with PP caused a significant increase of ammonia concentration in fish blood, however, it was restored to initial values within 24 h (Fig. 1c).

No changes were observed in Ca blood concentration, as well as in ALKP activity.

Detailed effects of PP anaesthesia on the blood plasma biochemical profile of carp are given in Table 3.

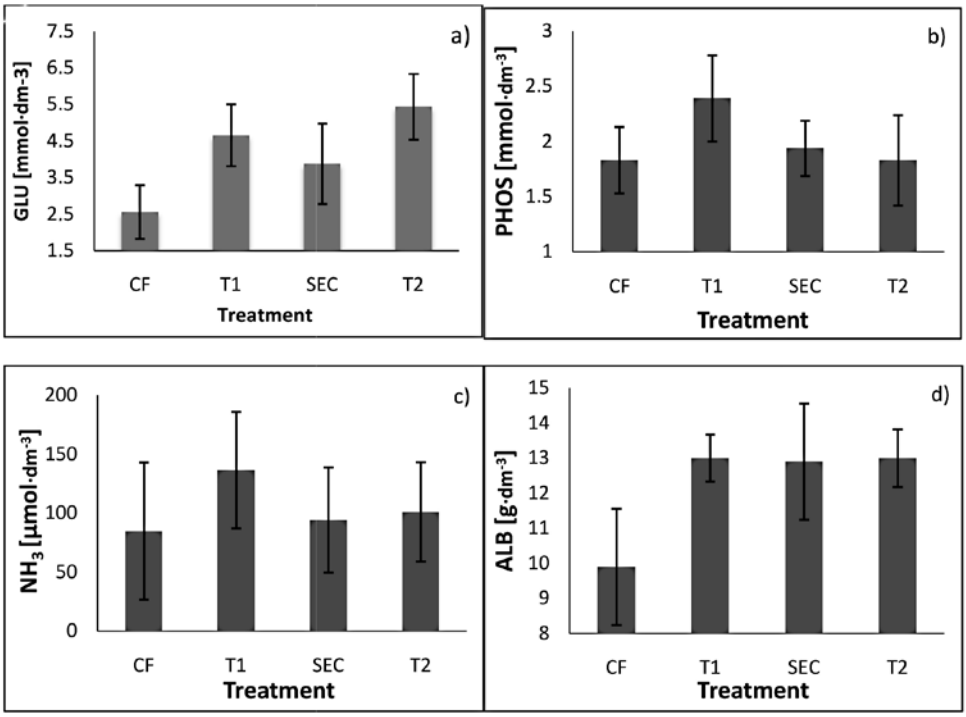

Fig. 1. Blood indices of common carp exposed to 1-phenoxy-2propanol anaesthesia
GLU – glucose, PHOS – inorganic phosphates, NH3– ammonia, ALB – albumin

Table 3. Changes in biochemical blood indices of common carp during PP anaesthesia.

Indices	Treatment			
	CF	T1	SEC	T2
TP [g·dm^{-3}]	27.50 ± 2.07^{a}	28.70 ± 2.58^{a}	29.30 ± 3.56^{a}	29.40 ± 2.46^{a}
ALB [g·dm^{-3}]	9.90 ± 1.66^{a}	13.00 ± 0.67^{b}	12.90 ± 1.66^{b}	13.00 ± 0.82^{b}
GLOB [g·dm^{-3}]	17.60 ± 1.51^{a}	15.70 ± 2.11^{b}	16.40 ± 2.22^{a}	16.40 ± 2.07^{a}
ALKP [µkat·dm^{-3}]	0.29 ± 0.09^{a}	0.21 ± 0.08^{a}	0.27 ± 0.12^{a}	0.26 ± 0.12^{a}
TRIG [mmol·dm^{-3}]	1.66 ± 0.36^{a}	1.80 ± 0.54^{a}	1.99 ± 0.67^{a}	1.72 ± 0.62^{a}
Ca [mmol·dm^{-3}]	2.04 ± 0.09^{a}	2.00 ± 0.17^{a}	2.04 ± 0.15^{a}	2.08 ± 0.20^{a}

Groups with different alphabetic superscripts differ significantly at $P < 0.05$ (ANOVA)
TP – total protein, GLOB – globulin, ALB – albumin, TRIG – triacylglycerols, ALKP – alkaline phosphatase, AST – aspartate aminotransferase, Ca – calcium, CF – control fish, SEC – stress exposed control fish, T1 – treatment 1 fish, T2 – treatment 2 fish, PP – 1-phenoxy-2-propanol

Discussion

Analysis of the haematological profile is an important tool that can be used as an effective and sensitive index to monitor physiological and pathological changes occurring in the fish organism. However, normal values of haematological indices differ greatly from one species to another (Landis et al. 2005; Antache et al. 2014). According to Caldwell

and Hinshow (1994) and Iwama (1998), sudden increase in the number of circulating erythrocytes as well as increase of PCV and Hb concentrations may be due to the release of red blood cells from the spleen. The PP anaesthesia resulted in a significant, almost two-fold increase of RBC count in anaesthetized fish. This increased level was maintained for 24 h. However, even higher erythrocyte count was noted in SEC fish compared to the T2 group. Significant decrease of MCV was also observed in anaesthetized fish compared to the control group. However, the mean MCV values were relatively higher than the ones reported by other authors (Velíšek et al. 2007a). Also, the significant increase of Ht level immediately after anaesthesia (0.37 ± 0.02) could occur due to the high level of RBC. However, it returned to the control level (0.28 ± 0.02) within 24 h after exposure to PP.

It is believed that leukopenia commonly occurs during the physiological response to acute stressors in fish (Wedemayer 1970; McLeay and Gordon 1977; Wedemeyer et al. 1983). Increased apoptosis of lymphocytes results from a rise of the cortisol level during a stress reaction (Wyets et al. 1998). The PP anaesthesia caused a significant decrease of the lymphocyte count in exposed fish. On the other hand, Velíšek et al. (2007a; 2007b) did not observe any changes in rainbow trout, carp, or in sheatfish exposed to 2PE.

Analysis of the biochemical blood profile is one of the most valuable methods available to modern diagnostics and can provide important information about the effects of anaesthetics on the fish organism (Iwama et al. 1989; Anver Celik 2004; Kristan et al. 2012). Under stress, the body of the fish emits immediate responses recognized as primary and secondary responses. The primary response is the perception of an altered state by the central nervous system (CNS) and the release of the stress hormones cortisol and catecholamines (adrenaline and epinephrine) into the bloodstream by the endocrine system (Randall and Perry 1992). Secondary responses occur as a consequence of released stress hormones (Barton and Iwama 1991) and are revealed e.g. as an increase of glucose and ammonia contents and as a decrease of the total protein concentration. Jahanbakhishi et al. (2012) report that an increased plasma glucose content during stress reflects the release of catecholamines and glucocorticoids from adrenal tissues of fish.

Blood plasma glucose concentration increased almost two-fold in fish of groups T1 (4.66 ± 0.85 $mmol \cdot dm^{-3}$) and T2 (5.44 ± 0.90 $mmol \cdot dm^{-3}$) in comparison to group CF (2.56 ± 0.73 $mmol \cdot dm^{-3}$). This indicates that the treatment used caused acute stress in experimental carp. The increase of glucose concentration was also observed in the gilthead bream (Ortuño et al. 2002), kelp grouper (Park et al. 2008), rainbow trout (Velíšek et al. 2011), and Senegalese sole (Weber et al. 2011) exposed to 2PE. However, Velíšek et al. (2004) did not observe any changes in glucose concentration in carp anaesthetized with 2-PE.

It is said that under prolonged stress, fish can mobilize fat stores and protein to meet an increased demand for energy necessary to sustain their increased physical activity (Iwama et al. 1986; Govindon et al. 1994). No differences in TRIG and TP contents were found between controls and fish exposed to PP in our experiment. However, a significant increase of ALB concentration was observed in all exposed fish. Velíšek and Svobodová (2004a,b) did not observe any changes in TP contents in rainbow trout and carp but they also found changes in ALB concentration in blood of rainbow trout exposed to 2PE. Blood analysis revealed increased concentration of ALB in sheatfish exposed to 2PE (Velíšek et al. 2007b).

It has been proved that respiratory acidosis is followed by an increase of phosphate and calcium concentrations and ammonia autointoxication (Ghosh and Joshi 2008; Gomułka et al. 2015). Increased ammonia content can also occur due to deamination of amino acids, especially glutamate, during extended energy production (Smutná et al. 2002). During our experiment, a significant increase of ammonia and phosphate concentrations was observed in T1 fish compared to controls. However, both ammonia and phosphate returned

to their initial concentrations within 24 h after exposure to PP. No changes in PHOS and NH_3 concentrations were found in the rainbow trout and common carp (Velíšek and Svobodová 2004a,b; Velíšek et al. 2011), sheatfish (Velíšek et al. 2007a) and perch (Velíšek et al. 2009) exposed to 2PE.

Our previous results (in press) showed that PP can be used as an efficient anaesthetic for common carp. However, the biochemical and haematological blood profile of common carp exposed to PP indicates that moderate, temporary stress occurred in fish exposed to PP. Moreover, changes caused by PP appear to be more severe than those reported in carp by Velíšek and Svobodová (2004a) as caused by 2PE. Thus, we recommend special care during the anaesthetizing of common carp with PP. Further research is needed to develop safe and effective procedure of anaesthesia with PP in fish.

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