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Original article

Pharmacokinetics of propofol in rainbow trout following bath exposure

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Abstract

Propofol, 2,6-diisopropylphenol, seems to be a good candidate as a fish anaesthetic, however, no study regarding propofol influence on fish has yet been reported. The aim of this study was to examine propofol pharmacokinetics in rainbow trout (*Oncorhynchus mykiss*) following bath exposure. Fish (n = 100) were exposed to an aqueous propofol bath at 12°C and 17°C; propofol concentration in the bath was 10 mg L^{-1}. Plasma concentration-time profiles were determined using LC-MS, and pharmacokinetic parameters were calculated. Propofol was absorbed quickly at both temperatures. Its concentration reached 13.8±2.7 μg mL^{-1} and 16.1±2.1 μg mL^{-1} at 12°C and 17°C, respectively, during the first minute of exposure. Blood plasma propofol decreased rapidly to 6.8±0.7 μg mL^{-1} and 6.3±2.2 μg mL^{-1} at 12°C and 17°C respectively, during the first 10 minutes of the recovery. The half-life time of propofol was 1.5 h and 1.1 h at 12°C and 17°C, respectively. We found propofol anaesthesia in trout effective and safe. However, it caused a gradual decrease of respiratory rate, and therefore a specific anaesthesia protocol should be developed.

Key words: 2,6-diisopropylphenol, anaesthesia, half-life time, LC-MS analysis, micro liquid-liquid extraction, *Oncorhynchus mykiss*

Introduction

Anaesthesia is believed to be an indispensible procedure in modern, intensive fish production. It facilitates many breeding operations, reduces stress in fish and thus also reduces potential losses caused by manipulation. It is recommended for any procedure causing pain or stress to fish. Anaesthesia is of particular importance during fish spawning, due to the usually high value and great size of spawners. Many chemicals show anaesthetic properties in fish (Bell 1967, Brown 1988). MS-222 (Baudin 1932), metomidate (Malmstroem et al. 1993, Mattson and Riple 1989, Olsen et al. 1995), etomidate (Amend et al.

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1982, Kazun and Siwicki 2001) and clove oil (Anderson et al. 1997, Keene et al. 1998) are probably the most frequently used worldwide. However, no one agent is suitable for all fish species and applications, and thus there is a demand for new, effective and safe anaesthetics for fish.

The evaluation of candidate anaesthetic agents usually involves efficiency (Marking and Meyer 1985) and toxicity assessment (Gilderhus 1990, Limsuwan et al. 1983a, Marking 1967). Anaesthetic influence on fish stress (Limsuwan et al. 1983b, Olsen et al. 1995) and biochemical and haematological indices (Gomułka et al. 2008, Velisek et al. 2006) have also been reported. There is a little information on the pharmacokinetics of anaesthetics in fish (Meinertz et al. 1996, Stehly et al. 1998, Guénette et al. 2007, Kiessling et al. 2009).

Propofol (2,6-diisopropylphenol) is an anaesthetic agent widely used for inducing anaesthesia in humans (Barash et al. 2009) and some mammal species (Flecknell 2009). Guenette et al. (2008) found that it anaesthetises frogs *Xenopus laevis* effectively. Fleming et al. (2003) found that propofol induced anaesthesia quickly in Mexico Gulf sturgeon. Miller et al. (2005) used propofol for anaesthetising a spotted bamboo shark. Propofol was also reported as an effective and safe agent for Siberian sturgeon *Acipenser baerii* (Gomułka 2009). Our preliminary study showed that propofol seems to be a good candidate for the induction of general anaesthesia in fish.

The aim of the study presented in this paper was to examine propofol pharmacokinetics in rainbow trout *Oncorhynchus mykiss* following fish exposure to a water propofol solution.

Materials and Methods

Chemicals

Diprivan (Pharma Zeneca), the commercial propofol preparation for intravenous injections in humans, was used to anaesthetise rainbow trout. Analytical standards of propofol (2,6-diisopropylphenol) and thymol (2-isopropyl-5-methylphenol) were purchased from SigmaAldrich (Poznan, Poland). Acetonitrile, methanol and cyclohexan were of LC-MS grade (Merck, Warsaw, Poland).

Equipment

An Agilent Technologies 1290 Infinity ultra-high performance liquid chromatograph (UHPLC) coupled to an Agilent Technologies 6460 triple quadrupole mass spectrometer (QQQ) equipped with an Agilent Jet Stream ion source was used for propofol determination.

Animals, exposure procedure and blood sampling

Healthy rainbow trout (n = 100) (*O. mykiss*) were acquired from a commercial fish farm (Czarci Jar Fish Farm, Drwęck, Poland). Exposure of the fish to propofol and subsequent blood sampling were carried out on the farm to avoid the stress induced by transportation and the change of water quality parameters. All fish were exposed to 1.0 ml L^{-1} of Diprivan (10 mg L^{-1} of propofol). The concentration was chosen in a preliminary study.

Experiments were carried out in spring (May) when the water temperature reached 12°C and in early summer (June) at a water temperature of 17°C. The fish mean length was 31.2 (±2.5) and 24.8 (±1.8) cm and the mean body weight was 422.8 (±77.2) and 244.1 (±51.3) g for the May and June study subset, respectively. The exposure procedure is described in detail in Table 1.

Table 1. Time regime of experimental exposure and recovery of rainbow trout to propofol solution. For each time point n=5.

Time point	Exposure time (min)	Recovery time (min)
Control	0	0
E1	1	0
E2	2	0
E3	3	0
E4	6	0
E5	12	0
ER1	12	10
ER2	12	30
ER3	12	60
ER4	12	180
ER5	12	360

Fish were fasted for 24 hours before the experiment. Each fish was individually captured from a rearing tank and moved to a 50 L polycarbonate tank filled with 30 L of an aqueous propofol solution, and equipped with a system ensuring the continuous bath aeration. A fresh propofol solution was used after the exposure of five fish. For time points E1 to E5 (Table 1) blood sampling was done immediately after the exposure. Fish from the remaining experimental groups (Table 1) after 12 min of exposure to propofol were moved to 50 L polycarbonate flowtrough tanks supplied with farm water continuously. The tanks

were covered with lids to prevent escape. The fish were blood sampled at defined recovery times (Table 1). After blood sampling, fish were placed in flow-through tanks supplied with farm water and observed for 48 hours. Water temperature during exposure and recovery was 12.2°C (±0.2) and 17.3°C (±0.4) in spring and summer subset experiments respectively.

Blood was sampled from the caudal vessels using syringes covered with haeparine lithium salt 50 µl ml^{-1} (Medlab Products, Raszyn, Poland) to prevent blood coagulation. Approximately 2.5 ml of blood was collected and transferred to haematological tubes and immediatelly centrifuged (StatSpin centrifuge, Idexx Inc) at 15,800 rpm (12,000 g) for 90 seconds. Plasma aliquots were transferred to Eppendorff tubes and stored at 21°C untill all samples were collected. Plasma samples were then transported to the laboratory and stored at -70°C.

Propofol extraction

To 200 µL of plasma, 4 µL of thymol solution in methanol (100 µg mL^{-1}) and 100 µL of phosphate buffer (pH 4.8) were added. Samples were vortexed for 1 min and then 1 ml of cyclohexane was added and samples were shaken for 5 min. After 3 min centrifugation at 3000 rpm the organic layer was collected (0.8 mL), and cyclohexane was evaporated under a gentle nitrogen stream. Dry residues were dissolved in 100 µL of 20 mM acetic acid in acetonitrile:water (80:20 v/v) solution and the samples were subjected to LC-MS analysis. Extraction of all samples was carried out in triplicate.

LC-MS analysis

Determination of propofol was carried out by UHPLC-QQQ in the presence of thymol as an internal standard. The separation was achieved on Zorbax Eclipse Plus C18 Rapid Resolution HD 2.1 x 50 mm, 1.8 µm column (Agilent Technologies). The mobile phase gradient from 60% to 95% B over 3 min with 1.5 min post run was applied, solvent A: 20 mM acetic acid in water, B: acetonitrile. Mobile phase flow was 0.3 ml min^{-1}. Compounds were ionized in electrospray (ESI). The detection was carried out in a negative SIM mode in three time segments: from 0-0.9 min the LC stream was diverted to waste and no MS data were collected; from 0.9-1.6 min ions of m/z 149.1 for thymol and from 1.6 to 3 min ions of m/z 177.1 for propofol were monitored with a fragmentor set at 120V and 100V for propofol and thymol, respectively. Injection volume was 5 µl. Instrument control and data acquisition were carried out by Agilent MussHunter Acquisition software v.B.03 and the data were analyzed by Agilent MussHunter Quantitative Analysis software v.B.03.

Pharmacokinetics

Plasma pharmacokinetic parameters of propofol were calculated using a non compartmental method (Rowland and Tozer 1995). The linear trapezoidal rule was used for the calculation of the area under the curve from time 0 to the last measurable concentration value (AUC_{0-t}). The mean plasma propofol concentrations for each time point (experimental group) were used for the above calculation. The terminal rate constant of elimination (k_{el}) was calculated as:

$$k_{el} = \{LN(C_{max}) - LN(C_{last})\}/ (t_1-t_0);$$

where: C_{max} is the mean plasma propofol concentration after 12 minute exposure, C_{last} – the last measured concentration after 6 hours of recovery, ($t_1 - t_0$) – time of recovery expressed in hours.

The terminal elimination half-life ($T_{1/2}$) was calculated using the $0.693/k_{el}$ formula. The area under the curve extrapolated to infinity (AUC_∞) was calculated as follows:

$$AUC_\infty = AUC_{0-t}\text{-}t + C_{last}/k_{el}.$$

Statistical analysis

All data, unless specified otherwise, are presented as the mean value ± standard deviation.

The Kruskal-Wallis test (non-parametric ANOVA) and Mann-Whitney test were used in the analysis of propofol blood concentration within each temperature data set. Two-way analysis of variance (ANOVA) was used to analyse differences in both uptake and removal between temperature data sets. The significance level was set as $p \leq 0.05$.

Results

Propofol anaesthesia. Anaesthesia stage IIIa (Siwicki 1984) was achieved within 2 min 43 s (±16 s). No significant temperature influence was observed. Propofol affected the rate of respiratory movement of the trout. Respiration gradually decreased during the exposure. All animals recovered from anaesthesia and no animal deaths occured within 48 hs.

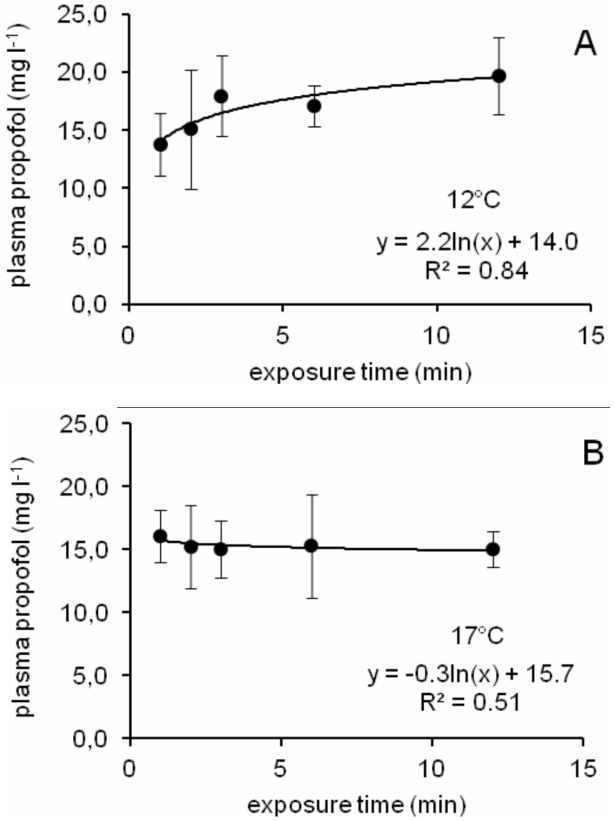

Fig. 1. Mean propofol plasma concentration values during bath exposure of trout at 12°C (A) and 17°C (B). For each time point n=5. Best fit regression lines and formulas are provided.

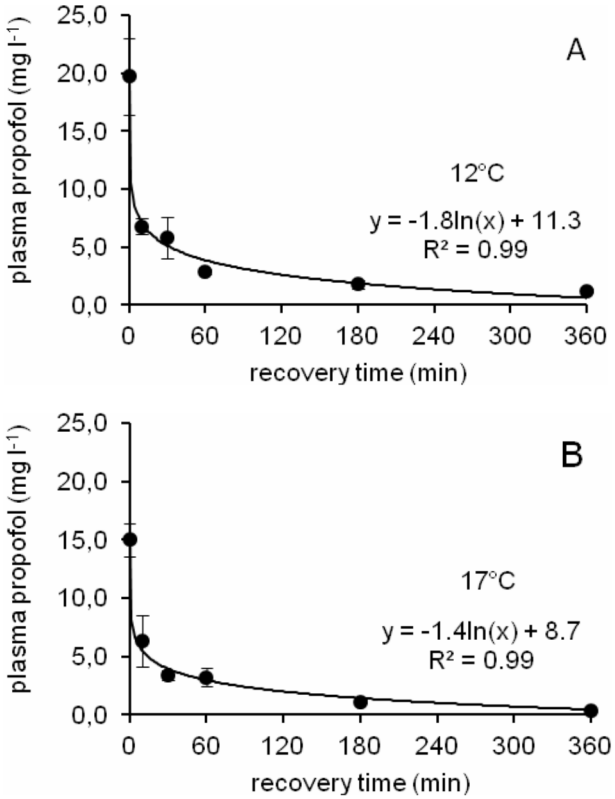

Fig. 2. Mean propofol plasma concentration values during trout recovery at 12°C (A) and 17°C (B). For each time point n=5. Best fit regression lines and formulas are provided.

Propofol determination. Micro liquid-liquid extraction (LLE) followed by ultra-performance liquid chromatography – mass spectrometry (LC-MS) assay was developed for the determination of propofol in rainbow trout plasma. This method enabled a convenient and efficient extraction of the anaesthetic from 200 µL of plasma aliquots. Under applied UHPLC conditions propofol eluated at 1.93 min and thymol, used as an internal standard, at 1.34 min. Agilent Jet Stream thermal gradient focusing technology (Jet Stream ESI source) provided higher sensitivity than Atmospheric Pressure Chemical Ionisation source (APCI) and was selected for the anaesthetic determination. Since the efficiency of propofol and thymol product ions formation was very low, the quantification was carried out in a selected ion monitoring (SIM) mode. $[M-H]^{-1}$ ions were monitored, and the m/z of molecular ions were 177.1 and 149.1 for propofol and thymol, respectively. For calibration, propofol-free plasma aliquots were spiked with propofol and thymol and samples were then subjected to extraction and LC-MS analysis. 9-point calibration at a range of 0.25 – 30 µg mL^{-1} with thymol as an internal standard was performed. A 1/x weighted first-order fit was used. The linearity of the calibration curve was excellent, and the coefficient of determination (r^2) was 0.996. Method limit of quantification was 0.1 µg mL^{-1}. Method repeatability (RSDs) was below 4% (n=11).

Propofol uptake. Propofol was rapidly absorbed both at 12°C and 17°C, and propofol plasma concentration reached 13.8 µg ml^{-1} (±2.7) and 16.1 µg ml^{-1} (±2.1), respectively, during the first minute of the exposure. Mean propofol plasma concentrations were not significantly different ($p>0.05$) between time points whithin the same temperature, except for the first time point at 12°C, where the propofol concentration was significantly lower compared to the remaining time points at this temperature. The drug concentration was at the same level from the 1st to the 12th minute of exposure at a temperature of 17°C (Fig. 1B). It rose slightly during exposure at 12°C and reached a maximum value of 19.7 µg ml^{-1} (±3.3) after 12 minutes of exposure (Fig 1A).

There were no significant differences of propofol concentrations between temperature groups compared at the same time points except for the last one, when the propofol plasma concentration was significantly higher at 12°C ($p<0.05$).

Propofol removal. Propofol concentration in trout

Table 2. Estimated pharmacokinetic parameters for propofol in rainbow trout blood plasma at 12°C and 17°C. Cmax – maximum measured concentration.

Exposure temperature (°C)	C_{max} (μg ml^{-1})	k_{el}	$T_{1/2}$ (h)	$AUC_{(0-t)}$ (μg h ml^{-1})	AUC^{∞} (μg h ml^{-1})
12	19.7	0.467	1.5	15.6	18.2
17	15.0	0.615	1.1	11.7	12.3

blood plasma decreased quickly to 6.8±0.7 μg ml^{-1} and 6.3±2.2 μg ml^{-1} at 12°C and 17°C, respectively, during the first 10 minutes of recovery (Fig. 2). These two values were not significantly different ($p>0.05$).

The rate of propofol elimination was higher at a water temperature of 17°C (Table 2) and the calculated terminal rate constant of elimination (k_{el}) was 0.467 and 0.615 for 12°C and 17°C, respectively. The last measured propofol concentration (after 6 hours of recovery) at 12°C was 1.2±0.2 μg ml^{-1} and was significantly higher ($p<0.01$) than that measured at 17°C (0.4±0.1 μg ml^{-1}).

The basic pharmacokinetic parameters of propofol are presented in Table 2.

Significant ($p<0.01$) interaction between temperature and propofol plasma concentration during the recovery was revealed by ANOVA.

Fish weight has not influenced on propofol blood concentration.

Disscussion

Propofol, used in the form of a water bath, produced safe anaesthesia for rainbow trout. No animal deaths were noticed 48 hs after exposure in both tested temperatures. The chosen concentration (10 mg of propofol per liter) can be recomended for this fish species. However, propofol strongly decreased the rate of gill ventilation with time of exposure, and thus longer exposure may be dangerous, probably leading to severe hypoxia. According to Ross (2001) anaesthetics can produce progressively deeper anaesthesia (even during recovery in anaesthetic free water) due to anaesthetic concentration increase in the brain and muscle despite its blood equilibration. A relatively stable plasma level of propofol was achieved during the first minute of rainbow trout exposure in our study. However, anaesthesia occurred after approximately 2 min and 43 s, and a progressive decline in respiratory rate was noticed. The above findings are in agreement with Ross (2001) data.

Propofol was rapidly absorbed from the water bath (Fig. 1). Anaesthetic plasma concentration was equal to about 1.5 times of the bath concentration from the 1st to 12th minute of exposure at 17 °C. Stehly et al. (1998) found the concentration of benzocaine in trout plasma to be as high as 1.54 μg mL^{-1}, 1.46 μg mL^{-1} and 1.22 μg mL^{-1} after 240 min of exposure to initial 1 mg L^{-1} of benzocaine at a water temperature of 6°C, 12°C and 18°C, respectively (from 150 min to 240 min plasma benzocaine concentration was equilibrated and approximately 2.4 times higher than bath benzocaine concentration). Guenette et al. (2007) studied the pharmacokinetics of eugenol in rainbow trout. He found the maximum blood concentration of the drug to be as high as 10.53 μg mL^{-1} following exposure to 75 mg L^{-1} of eugenol for 15 min at 4°C water temperature (ca. 0.14 of the bath concentration).

The rate of elimination of propofol from trout blood was similar to those reported for benzocaine. The half-life time for propofol was 1.5 h and 1.1 h for water temperatures of 12°C and 17°C, respectively. Stehly et al (1998) found half-life times for benzocaine as 1.0 h, 0.6 h and 0.7 h for 6°C, 12°C and 18°C, respectively. According to Guenette et al. (2007), the plasma half-life time of eugenol was 12.1 h and the authors suggest its potential tendency to accumulate in trout tissues.

Conclusions

We found propofol anaesthesia in trout effective and safe; however, during propofol anaesthesia the respiratory rate decreases gradually, and therefore a specific detailed anaesthesia protocol should be developed.

The rates of both propofol absorption and elimination are high and comparable to those observed for benzocaine.

As temperature affects propofol elimination from trout blood, a longer time of recovery should be expected at lower water temperatures. A more detailed study on propofol elimination from trout blood and tissues in low temperatures is needed.

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