EFFECT OF IBUPROFEN ON THE SWIMMING PATTERN OF *Cyprinus carpio*

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ABSTRACT

Ibuprofen is a non-selective inhibitor of cyclooxygenase with antiinflammatory, antipyretic and analgesic activity; its mechanism of action involves the inhibition of prostaglandins biosynthesis. Its presence in water-bodies of peri-urban areas as a result of domestic discharges is highly frequent. The aim of this paper was to evaluate the impact of drug exposure on some parameters of the swimming behaviour of the common carp under laboratory conditions. Semi-static bioassays were carried out with juveniles of *Cyprinus carpio* as test organisms. Assays comprised three periods: acclimation (7 days in tap water [TW]), control (4 days in TW) and exposure (13 days in TW + 100 µg.L^{-1} ibuprofen). During control and exposure, the parameters were registered to calculate swimming activity (as Relative Activity Index; I_a) and swimming velocity. The drug inhibited the swimming activity with symptoms corresponding to *Hypo-Hyperactivity Syndrome of* Drummond and Russom; swimming velocity was slightly reduced. There were no lethal effects registered in any case. It was then concluded that, under assay conditions, I_a represents a suitable behavioural biomarker for assessing ibuprofen effects on swimming activity of *Cyprinus carpio*.

KEYWORDS: *Cyprinus carpio*, ibuprofen, bioassays, swimming activity, swimming velocity

1. INTRODUCTION

Drugs are produced and used in variable amounts which may reach thousands of tonnes a year [1]. Many of them (as metabolites and/or unaltered) reach the water-bodies either discharged through the sewage system, or having gone through the treatment plant unmodified. The discharge of these effluents into urban and peri-urban watercourses results in the chronic exposure of aquatic organisms to these substances [2-4]. Non-steroidal anti-inflammatory drugs (NSAID), in particular, inhibit the cyclooxygenase which catalyzes the biosynthesis of prostaglandins, partially involved in the genesis of pain and swelling. This inhibition is responsible for their analgesic and antiinflammatory effect. Ibuprofen is one of the most commonly used NSAIDs. It is a drug resistant to degradation and a persistent pollutant found in surface waters, since it is not degraded during sewage treatment [5]. Concentrations detected in effluents of surface sewage waters are found in order of ng and µg.L^{-1} [6, 7].

Available evidence shows there is bioaccumulation of these products in fish [8-10]. There are toxicological studies of effluents from sewage treatment plants and discharges from pharmaceutical companies documenting important changes in diverse aspects of swimming activity in fish exposed to these media.

The aim of this paper was to assess the impact of ibuprofen in sublethal concentrations on two parameters of swimming behaviour in the common carp (*Cyprinus carpio*) under laboratory conditions.

2. MATERIALS AND METHODS

2.1 Test species

Test organisms were juveniles of *Cyprinus carpio* (weight: 4.9 ± 0.4 g; total length: 7.0 ± 0.4 cm; n = 12). The fish were obtained from a commercial supplier and had never been exposed to pollutants. Prior to use, the fish were acclimated to laboratory conditions (21.1 ºC, 12:12 light:dark cycle, permanent aeration) for at least 15 days in glass aquaria under continuous flow through a system with tap water (TW).

2.2 Experimental bioassays

Three semi-static bioassays were carried out, with medium renovation every four days under the same environmental conditions as those in acclimation. The experimental device was that of Eissa *et al.* (2006) [11]. Each glass aquarium was fitted with 40 infrared (IR) sensors and emitters on the external wall, and contained one fish and 12.8 L of TW. The location of the fish was recorded

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when the IR beam was interrupted; the signals were recorded automatically and continuously in real-time at a rate of one per second. A computer-based data acquisition system was used to collect the data which were then analyzed using specially designed software. The data collection system works in parallel and has a control panel in each aquarium. These data are transferred via a serial connection to the controller that converts them into serial data, which are received by a PC and stored in a data base. The data used to estimate fish activity were the number of times each IR beam was blocked. In order to avoid variations in locomotive activity of animals due to circadian rhythms [12, 13], recordings were carried out daily from 12 am to 2 pm.

Assays comprised three consecutive periods: acclimation (7 days in tap water [TW]), control (C) (4 days in TW) and exposure (E) (13 days in TW + 100 µg.L^{-1} ibuprofen); during E, the medium was renewed every four days. Fish were fed daily with commercial fish food. Feed was administered at the end of the recording period.

The following basic physicochemical parameters of water were monitored daily: pH, dissolved oxygen, conductivity (HqD Field case Hach); hardness (test kit, Merck 108039) and temperature. Data were expressed as means ± SEM. Swimming Activity Index (A_i) and Swimming Velocity (V) were calculated from the data registered during control and exposure periods.

1) Swimming Activity Index (Ai).

It was calculated from the average number of the total moves recorded daily over 2 hours at each one of the experimental periods (C, E) divided by the number of total moves recorded on each particular day *i* of the period, where *i* = experimental day:

$$Ai = \frac{\text{average number of total moves registered in the period (C or E)}}{\text{number of total moves on day } i}$$

The *Ai* indicates changes in fish activity. The value of $A_i = 1$ denotes no effect while $A_i < 1$ and $A_i > 1$ denote increases or decreases in fish activity, respectively [11, 14, 15].

2)Swimming Velocity (V).

It was calculated from daily records, determining the distance swum and time spent swimming [16], using the formula:

$$V = dn - \frac{m}{dt}$$

where, *V* is *swimming velocity* (cm.s^{-1}); *n* is the *starting* point; *m* the *arriving* point, and *t* the time between both points (seg).

An average of mean daily values was calculated for each period. Data of fish activity were analyzed separately for each one of the experimental periods (C and E).

2.3 Statistical Analyses

Swimming Activity Index was assessed daily through Wilcoxon signed-rank test [17]. Swimming velocity was assessed through t-test; assumptions of normality and homoscedasticity were tested using the Kolmogorov-Smirnov and the Bartlett tests, respectively [18]. The significance level was set at 95% ($P < 0.05$). Statistical calculations were performed using the Infostat software.

3. RESULTS AND DISCUSSION

3.1 Physicochemical parameters of the bioassay media

Table 1 shows the mean values of the water quality parameters for each experimental period for the three assays that were carried out. The levels of dissolved oxygen were suitable, resulting in no additional stress.

TABLE 1 - Physicochemical parameters of the bioassay media of Control (TW) and Exposure (TW+100 µg.L^{-1} Ibuprofen). Data as means ± SEM; number of measurements in parenthesis.

Parameter	Control (C)	Exposure (E)
pH	8.83±0.02 (12)	8.85±0.02(54)
Dissolved oxygen (mg.L^{-1})	8.27±0.06 (12)	8.09±0.07(54)
Hardness (mM $CaCO_3$)	0.80±0.06 (12)	0.84±0.01(54)
Conductivity (µS.cm^{-1})	957.23±2.31(12)	965.85±1.75(54)
Temperature (ºC)	21.6±0.1(12)	21.6±1.7(54)

3.2 Swimming parameters

3.2.1 Swimming Activity Indexes

Figure 1 shows the change sequence in Ai registered in a typical assay. It can be seen that during control (days 1 to 4), Ai was constant in threshold values (Ai = 1). From the first day of exposure, Ai began oscillating, showing changes in swimming activity. Wilcoxon signed-rank test proved significance ($p<0.01$), which is evidence of the deviations from threshold values registered prior to the introduction of ibuprofen (days 1 to 4 of exposure).

It can be observed that, during exposure to ibuprofen, the follow-up of daily responses in Ai of *C. carpio* presented oscillations that are correspondent with the *Hypo-Hyperactivity Syndrome* (Hypo-AS e Hyper-ASA) observed by Drummond and Russom (1990) [19]. The two peaks that can be seen in Ai may be related to the degradation of ibuprofen in the media, since according to bibliography, 90% of ibuprofen is degraded within 6 days [5].

3.2.2 Swimming Velocity

Although there was a decreasing tendency, there was no significant reduction in swimming velocity during exposure to ibuprofen.

It should be noted that there were no lethal cases registered while the assays lasted, which would allow us to infer that ibuprofen is subtoxic for juveniles of *Cyprinus carpio* at a concentration of 100 µg.L^{-1}. LC_{50} (24 h) for *Cirrhinus mrigala* was determined at 142 mg.L^{-1} by Saravanan *et al.* [20].

There is a variety of ways to quantitatively assess fish activity [21, 22], but Ai was used herein. This index re-

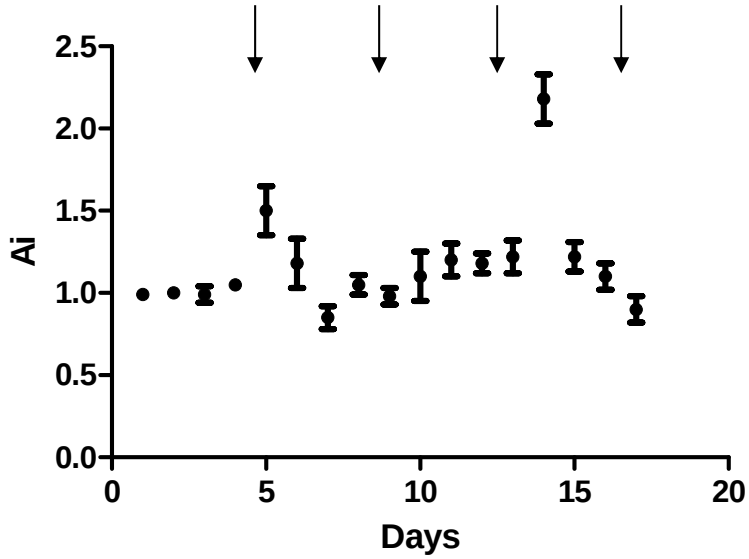

FIGURE 1 - Swimming Activity Index Ai (means ± ESM; control period: day 1 to 4; exposure period: day 5 to 17; the arrows indicate the time of medium renewal).

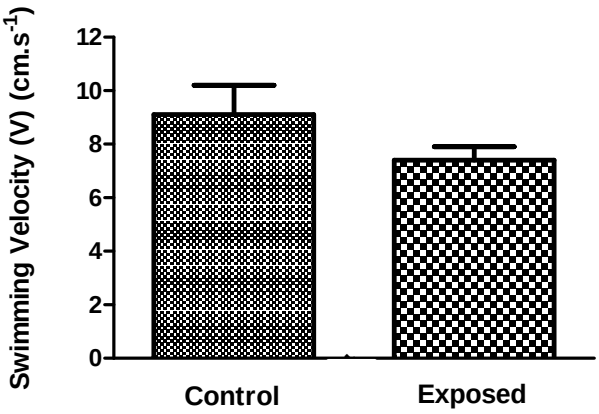

FIGURE 2 - Swimming Velocity (V) (cm.s^{-1}) as means ± ESM

sembles the mean lethal concentrations (LC_{50}) used to quantify responses in short-term assays: a low value of Ai denotes an increase in activity while a high value is an evidence of decreased activity, both secondarily to an exposure to a toxic pollutant [23]. In addition, the index avoids the use of large figures, and allows overcoming both large within-group variability in the responses and large differences between exposed and control animals [16].

A previous research carried out in our laboratory confirmed the usefulness of Ai as a biomarker of stress on fish exposed to pollutants. In assays on exposure to sublethal doses of cadmium, the index increased (the metal significantly decreased swimming activity) [16, 23]. The same happened in assays carried out with environmental samples from a polluted peri-urban river (Reconquista River, Buenos Aires); these changes were contrasted and validated with the chemical profiles [24].

David and Pancharatna (2009) [25] observed alterations in the swimming patterns of zebra fish exposed during their initial development to ibuprofen, as well as in their response to tactile and visual stimuli. This could be associated with malformations in the heart and fins, the retarded body development and the curved spine caused by early exposure to the drug.

Even though the dose was sublethal, it is worth highlighting that the concentrations of individual drugs that are expected in the media may be smaller than ours [26, 27], and as the ecotoxicity of altered environments can be different, we consider the mix of drugs or products of biological or environmental degradation (salinity, temperature, photooxidation, etc), their isomeric composition, etc. [28].

Monitoring carried out by Elorriaga *et al.* (2012) [29] on the sewage system discharges in diverse urban areas located on the southern bank of the Río de la Plata Estuary, and in lagoons in the Pampean region of Argentina, revealed that the concentration of ibuprofen reached values close to 10 µg/L. These were the first reports from our country that allowed the detection of pharmacological products being introduced in the local water-bodies, as these values were similar to those found in other freshwater environments. It is also important to mention the work of Valdes *et al.* (2014) [30]. They reported on the occurrence as well as the spatial and temporal variations of some common prescribed pharmaceuticals in the Suquía River basin (Córdoba, Argentina). Among 15 compounds analyzed, atenolol, carbamazepine and diclofenac were the most frequently detected.

4. CONCLUSIONS

Kolok *et al.* (1998) [12] and Kolok (1999) [31] found that individual variation associated with swimming performance are important and inevitable, even when the fish being studied are morphologically comparable and at the same stage of life. They concluded that the analysis of experimental data should be done through individual approximation, assessing the behavioural parameters of each animal before and after exposure to the toxicant.

Ibuprofen caused *Hypo-Hyperactivity Syndrome* on the carps, and a tendency to reduce swimming velocity. This may be due to the inhibition of prostaglandins affecting muscular contraction and, consequently, swimming ability [32].

Behavioural changes in fish as biomarkers of environmental stress are becoming growingly interesting for ecotoxicology assessment of pharmacological products [33]. Even restricted to the individual level, they can have significant consequences on more complex different levels of environmental organization that could be quantified.

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