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Purinergic signalling as a potential pathway for trichlorfon induced-inflammation and impairment of the immune response using freshwater silver catfish

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ABSTRACT

Adenosine triphosphate (ATP) and adenosine (Ado) are the main molecules of the purinergic signalling involved in toxicological mechanisms induced by pesticides. These molecules participate in the regulation of immune and inflammatory responses due to their interaction with purinereceptors in the extracellular medium. In the literature, the ATP and Ado are related with pro-inflammatory and immunomodulatory effects, respectively. Thus, the aim of this study was to evaluate whether the purinergic signalling can be considered a potential target for trichlorfon-induced inflammation and impairment of the immune response. Seric and splenic triphosphate diphosphohydrolase (NTPDase) activities using ATP as substrate were reduced at 22 mg/L after 48 h of exposure compared to the control group, while NTPDase activity using ADP as substrate was lower only in the serum of silver catfish at the same period. On the other hand, seric and splenic adenosine deaminase (ADA) activities and metabolites of nitric oxide (NOx) levels were increased at 22 mg/L after 48 h of exposure compared to the control group. The enzymatic activity of the purinergic signalling did not return to control levels after 48 h of recovery period in trichlorfon-free water. Based on these evidences, we concluded that trichlorfon interferes with the purinergic signalling, causing impairment of the immune and inflammatory responses by reducing ATP hydrolyses, possible leading to its accumulation in the extracellular environment. In addition, there is an increase in Ado desamination and possible reduction of it in the extracellular medium, leading to a self-sustained pro-inflammatory deleterious cycle. In summary, the purinergic signalling can be considered a potential target for trichlorfon-induced inflammation and impairment of the immune response in freshwater silver catfish at exposure to 22 mg/L.

1. Introduction

The use of organophosphorus compounds in aquaculture to control or eliminate ectoparasites is quite common in intensive production systems, sport fishery and commercial fish farming in order to improve quality and avoid economic losses (Hispano et al., 2016; Thing et al., 2016). In spite of numerous advantages, the excessive and incorrect use of pesticides represent an important environmental and public health problem worldwide, including for aquatic ecosystems (Baldissera et al., 2018a). The application of pesticides, as trichlorfon, may lead to the contamination of aquatic environments *via* different routes including spray drift, runoff and leaching, inevitably eliciting interruptions on aquatic food chain, and consequently, lost/shift of abundant natural species (Venturini et al., 2015; Uddin et al., 2016).

Trichlorfon (dimethyl, (2,2,2-trichloro-1-hydroxyethyl) phosphonate) is one of the most extensively used pesticide to control and treat freshwater and marine fish infested by *Dactylogyrus* sp., *Gyrodactylus* sp., *Urocleidoides* sp., *Trianchoratus* sp., *Argulus* sp., *Ergasilus* sp., *Lernea* sp., and *Trichodinas* sp. (Trujillo-González et al., 2018) due to its relatively low bioaccumulation and short-term persistence (Rao and Kavitha, 2004). Despite its short half-time life in the water (approximately 57 h) (Lopes et al., 2006), excessive amounts of trichlorfon often applied in fish and agriculture farm management and its residues (mainly the metabolite dichlorvos, 2–2,dichlorovinyl dimethyl

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phosphate) have been associated to severe toxicity for many fish species, as an inhibitor of acetylcholinesterase activity and histopathological alterations in gills of Nile tilapia (*Oreochromis niloticus*) (Guimarães et al., 2007), hematological alterations in common carp (*Cyprinus carpio*) (Chandrasekara and Pathiratne, 2005), oxidative damage in gills and liver of Tra catfish (*Pangasianodon hypophthalmus*) (Sinha et al., 2010) and goldfish (*Carassius auratus gibelio*) (Xu et al., 2012), metabolic alterations in muscle, liver and plasma of pacu (*Piaractus mesopotamicus*) (Venturini et al., 2015), and behavioral alterations linked with imbalance, slowness, paralysis and wink-beat convulsions of stinging catfish (*Heteropneustis fossilis*) (Mishra et al., 2014). However, trichlorfon possible toxic effects in fish immune system remains unknown, including its effects on the purinergic signalling, a pathway linked with impairment of immune and inflammatory responses in fish exposed to pesticides (Baldissera et al., 2018b).

The purinergic signalling, by extracellular catabolism of purinergic molecules, as adenosine triphosphate (ATP), adenosine diphosphate (ADP), and adenosine (Ado) acts through positive or negative signals that modulate the immune and inflammatory responses (Sévigny et al., 2015; Savio et al., 2018). This modulation is signaled by the interaction of purine nucleotides with specific purinereceptors present in the plasma membrane of immune tissues defining how and to what extent inflammatory cells should react through interactions with these specific receptors, as purinereceptors P2X7 and P1 (Junger, 2011; Burnstock, 2018). The spleen expresses a multi-enzyme complex found anchored in the cellular membrane of immune cells, which includes the enzymes NTPDase (triphosphate diphosphohydrolase), 5′-nucleotidase, and adenosine deaminase (ADA) (Di Virgilio and Vuerich, 2015). The conversion of ATP and ADP to adenosine monophosphate (AMP) is catalyzed by NTPDase, whereas 5′-nucleotidase catalyzes the degradation of AMP to Ado. Subsequently, Ado is deaminated into inosine by the action of ADA (Savio et al., 2016). Under physiological conditions, ATP is almost exclusively present in the intracellular environment, and practically negligible in the extracellular environment (Burnstock, 2007). However, under pathological conditions, as well as during pesticides exposure, ATP accumulates in the extracellular medium, and in sites of tissue injury and inflammation, acting as a damage-associated molecular pattern (DAMP), and behaves as a mediator of inflammation after its release by necrotic or apoptotic cells. Also, large amounts of ATP results in sustained P2X7 purinereceptor activation, leading to a self-sustained pro-inflammatory deleterious cycle (Burnstock, 2016; Savio et al., 2018). Several evidence have demonstrated that the purinergic signalling exerts an important role in the pathological mechanism linked to impairment of immune and inflammatory responses during exposure to pesticides, as observed using carbofuran, malathion (Senger et al., 2005), and thiamethoxam (Baldissera et al., 2018b). Thus, our hypothesis is that purinergic signalling can be a pathway involved in impairment of immune and inflammatory responses during trichlorfon exposure. Based on these evidences, the aim of this study was to evaluate whether the purinergic signalling can be considered a potential target of trichlorfon-induced inflammation and impairment of immune response using silver catfish as the experimental model.

2. Material and methods

2.1. Chemicals and water trichlorfon quantification

Trichlorfon (800 g/kg) was purchased in a Brazilian market with the commercial name of Masoten® (Bayer, Brazil). The ectoparasiticide was added to the test water at 11 and 22 mg/L prior to the beginning of the experiment based on the protocol stablished by Yonar et al. (2015), which observed its toxic effects in other freshwater teleost fish, as common carp. Also, both sublethal trichlorfon concentrations (11 and 22 mg/L) were selected based on its lethal concentration for the same species (LC_{50} = 61.98 mg/L).

The concentration of trichlorfon was determined in the water from the tanks at the beginning (0 h), 24 and 48 h of exposure, as well as 48 h after in trichlorfon-free water recovery span. For this, the water samples were filtered in 0.22 μm membrane filters and diluted for suitable injection on ultra-high performance liquid chromatography coupled to mass spectrophotometry (UHPLC-MS). The UHPLC-MS system (model Waters) was equipped with liquid chromatograph, MS detector type triple quadrupole (model Xevo TQ), interface/source of electrospray ionization, peak nitrogen generator, solvent controller system (binary pump system) to operate under gradients of high pressure, analytic column UPLC BEH C18 (50 × 2.1 mm, 1.7 μm) (Water, USA) and data acquisition system through MassLynx® 4.1 software (Waters, USA). Trichlorfon identification and quantification were performed based on monitored reactions. A mobile phase containing water and methanol (98:2, *v*/v) (A) and containing methanol (B), both constituted by 5 mmol/L of ammonium formate and formic acid at 0.1% (*v*/v) with a flow of 0.225 mL/min and 10 μL of injection volume in a total running time of 3 min was used to quantify the trichlorfon in water using a gradient elution method: 0 and 15 s (95% A; 5% B), 90 and 150 s (5% A; 95% B), and 156 and 180 s (95% A; 5% B). Trichlorfon concentration in water was shown in Table 1.

2.2. Fish maintenance and experimental design

Silver catfish juveniles (55.11 ± 5.25 g; 11.50 ± 1.65 cm) were collected for experimental purposes from a fish farm located in Southern Brazil. The animals were transported alive for the Fish Physiology Laboratory and maintained for acclimation in 250 L fiberglass tanks with continuous aeration and controlled water variables (temperature: 22 ± 0.5 °C; pH: 6.66 ± 0.2 and dissolved oxygen: 6.22 ± 0.52 mg/L) for seven days. The fish were fed to apparent satiation with commercial pellets (42% of brute protein; Supra®, Brazil) once a day and continuous feeding during the experimental period. Any uneaten food, feces and other residues were removed daily 60 min after feeding.

The fish were allocated inside tanks of 30 L with continuous aeration and exposed 0, 24 and 48 h to trichlorfon, followed by 48 h of recovery in trichlorfon-free water (54 fish; three replicates per concentration; six fish per replicate): 0.0 (control), 11 and 22 mg/L. For analyzes of 48 h of recovery in trichlorfon-free water, the animals were transferred to others cages in order to avoid possible interference of trichlorfon residues. Water quality variables (temperature, dissolved oxygen, pH, ammonia and non-ionized ammonia) were evaluated daily according the methodologies described below: dissolved oxygen and temperature were measured with a YSI oxygen meter (Model 75512,

Table 1
Trichlorfon concentration added in the water (11 and 22 mg/L) under experimental conditions at 0, 24 and 48 h of exposure, as well as 48 h after recovery in trichlorfon-free water.

Sample	Trichlorfon
0 h	
11	8.04 ± 0.57
22	18.10 ± 1.25
24 h	
11	3.78 ± 0.33
22	7.11 ± 1.62
48 h	
11	0.577 ± 0.11
22	2.09 ± 0.23
48 h of recovery span	
11	n.d.
22	n.d.

Values expressed as mean ± standard deviation. Limit of detection: 0.06 mg/L; limit of quantification: 5 mg/L. Note: n.d. (not detected).