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Toxicological responses on cytochrome P450 and metabolic transferases in liver of goldfish (*Carassius auratus*) exposed to lead and paraquat

Xiaoming Xu[a,b], Zhaojie Cui[a,*], Xinlei Wang[a], Xixin Wang[b], Su Zhang[b]

[a] School of Environmental Science and Engineering, Shandong University, Jinan 250100, China
[b] School of Municipal and Environmental Engineering, Shandong Jianzhu University, Jinan 250101, China

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ABSTRACT

As the producer of reactive oxygen species (ROS), both lead (Pb) and paraquat (PQ) can generate serious oxidative stress in target organs which result in irreversible toxic effects on organisms. They can disturb the normal catalytic activities of many enzymes by means of different toxicity mechanism. The changed responses of enzymes are frequently used as the biomarkers for indicating the relationship between toxicological effects and exposure levels. In this work, goldfish was exposed to a series of test groups containing lead and paraquat in the range of 0.05–10 mg/L, respectively. Four hepatic enzyme activities, including 7-ethoxyresorufinO-deethylase (EROD), 7-benzyloxy-4-trifluoromethyl-coumarin-O-debenzyloxylase (BFCOD), glutathione S-transferase (GST) and UDP-glucuronosyltransferase (UGT) were determined after 1, 7, 14, 28 days exposure. The results showed that the activities of EROD and BFCOD in fish were significantly inhibited in response to paraquat at all exposure levels during the whole experiment. Similarly, the inhibitory effects of lead exposure on BFCOD activity were found in our study, while different responses of lead on EROD were observed. There were no significant differences on EROD activity under lower concentrations of lead (less than 0.1 mg/L) before 14 days until an obvious increase was occurred for the 0.5 mg/L lead treatment group at day 14. Furthermore, lead showed stronger inhibition on GST activity than paraquat when the concentrations of the two toxicants were more than 0.5 mg/L. However, the similar dose and time-dependent manners of UGT activity were found under lead and paraquat exposure. Our results indicated that higher exposure levels and longer accumulations caused inhibitory effects on the four enzymes regardless of lead or paraquat stress. In addition, the responses of phase I enzymes were more sensitive than that of phase II enzymes and they may be served as the acceptable biomarkers for evaluating the toxicity effects of both lead and paraquat.

1. Introduction

For the persistent accumulation and high toxicity, lead (Pb) poisoning is consistently acknowledged to be one of the most concerned metal pollution through anthropogenic emission from the sewage of mining, industrial manufacture involved in lead compounds and irregular use of pesticides (Sorrentino et al., 2017). Although the severe situation has been partly alleviated with the utilization of non-lead substitutes, lead pollution is still widespread and difficultly preventable in environment (Wang et al., 2017; Dao et al., 2017). High levels of lead beyond guidelines were frequently detected in soil, sediments and many rivers near mines or industries (Karimi et al., 2017; Baltas et al., 2017; Kerr and Cooke, 2017). As a nonessential element in biological processes (Khan et al., 2016), the absorbed lead is highly toxic for humans and animals even at trace concentration. It can be coupled to erythrocytes and accumulated in tissues and organs through circulation (Feksa et al., 2012). Many researchers have reported that the highest accumulated concentration of lead in aquatic organisms exceeded the acceptable upper limit which presented potential risk for human health (Benali et al., 2017; Gu et al., 2017; Mariussen et al., 2017). Lead can inhibit the heme biosynthesis for tightly covalent binding to active site (-SH) of δ-aminolevulinic acid dehydratase (δ-ALAD) which synthesizes the molecule of porphobilinogen (PBG) (Dos Santos et al., 2016). Remarkably, lead can produce more reactive oxygen species (ROS) because of the excess δ-aminolevulinic acid (ALA) and targeted assault on antioxidant system, which break the equilibration between generation and elimination of ROS and do harm to cell membrane, protein denaturation and lipid peroxidation (Almeida Lopes et al., 2016; Shenai-Tirodkar et al., 2017). Some studies have reported that Pb^{2+} can block Ca^{2+} uptake through competing for the same sites in Ca^{2+} channels. This behavior results in the disorder of intracellular calcium homeostasis due to the inhibition of Ca^{2+} ATPase and Na^{+} /K^{+} ATPase

* Corresponding author.
E-mail address: cuizj@sdu.edu.cn (Z. Cui).

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(Alsop et al., 2016; Karri et al., 2016).

Paraquat (PQ) is one of the most popular dipyridyl herbicides for agricultural production all over the world, though quite a few countries have introduced relevant restrictions for its marketing and application. With the characteristic of fast-acting and little bioavailability to crops, low-cost paraquat is considered to be an available and popular herbicide in about 100 countries (Pizzutti et al., 2016). Because paraquat is large-scale used and highly soluble in water, it has an inevitable tendency to enter aquatic systems together with leaching and runoff from farmlands (Ling et al., 2017). Additionally, some reports have demonstrated that paraquat was directly used for the controls of hydrophyte and the concentrations of application range from 0.1 to 2 mg/L (Chuntib and Jakmunee, 2015). Although paraquat is generally considered to be safe for agriculture, a low dose of paraquat solution can cause the extremely harmful damage and high mortality mostly on account of acute lung injury (ALI) for human (Huang and He, 2017; Zhu et al., 2017). Definitely, a primary mechanism of paraquat poisoning is the generation of superoxide anion ($O_2^{\bullet -}$) during its redox cycling, which induces the detrimental oxidative stress as well as lead poisoning (Gravina et al., 2017). Furthermore, the concurrent oxidation of NADPH in the formation of the paraquat radical can disrupt many biochemical processes based on the involvement of NADPH (Díaz et al., 2016). Quite a few studies have been carried out to find the toxic effects and mechanisms of paraquat in plants and mammals (Heredia et al., 2015; Vidović et al., 2016; Moustakas et al., 2016), but there are not enough concerns for characterizing the toxicologic responses of paraquat in fish.

For guiding a definite correlation between pollutant levels and its toxic effects in organisms, various biomarkers of exposure and effects are developed and widely used to evaluate the toxic levels in experimental individuals after acute and chronic exposure to pollutants (Abarikwu, 2013; Jebali et al., 2013). All kinds of enzymes in organisms involve in nearly all of biological metabolism, including redox, hydrolysis, synthesis and so on. In this context, they were used as the preferred tools for indicating the cytotoxicity and physiological responses to xenobiotics.

Cytochromes P450 (CYP450) are admitted to the most important monooxygenase in liver mainly responsible for catalyzing the addition of functional groups to the exogenous toxicants during phase I reactions (Uno et al., 2012). Among the CYP450 superfamily, CYP1A and CYP3A subfamilies are intensively concerned because of their sensitive responses to drugs and environmental pollutants (Arellano-Aguilar et al., 2009). Acting as the catalytic probes, 7-ethoxyresorufin-O-deethylase (EROD) and 7-benzyloxy-4-trifluoromethyl-coumarin-O-debenzyloxylase (BFCOD) were applied to measure the activities of CYP1A and CYP3A enzymes, respectively (Zanger and Schwab, 2013; Ding et al., 2016). EROD has been established as the most current molecular biomarker for polycyclic aromatic compounds, especially benzo[a]pyrene (BaP) in aquatic monitoring (Fang et al., 2010; Lyons et al., 2011) and BFCOD has been proved to be an efficient parameter for the metabolism of pharmaceuticals (Ding et al., 2016; Steinbach et al., 2016). Numerous papers have studied the toxic effects of lead and paraquat poisoning with the help of many classical biomarkers, e.g., δ-aminolevulinic acid dehydratase (ALAD) and many enzymatic and non-enzymatic antioxidants (Chankova et al., 2014; Han et al., 2016; Fernández et al., 2015). However, we are unaware of the response on CYP450 enzymes in presence of lead or paraquat, especially in fish.

Glutathione S-transferase (GST) and UDP-glucuronosyltransferase (UGT) are essential enzymes with the function of catalyzing conjugation reactions during phase II biotransformation, which transform numerous hydrophobic and electrophilic organic compounds into water-soluble, less or non-toxic metabolites (Anjum et al., 2012). Therefore, increasing investigations have been carried out to find the biological responses on GST and UGT activities in organisms to evaluate the toxicity of numerous organic pollutants (Srikanth et al., 2013; Yang et al., 2013). Previous researches have reported that many metals can cause different influence on GST activity in fish, but more attention is emphasized on environmental monitoring of heavy metal pollution in water (Rajeshkumar et al., 2013; Saleh and Marie, 2016). Further knowledge is still lack for toxicological responses of lead exposure and accumulation in fish. Similarly, limited information relating to UGT effects was found in fish exposed to heavy metals and herbicides except for a large number of applications on the metabolism of drugs, phytochemicals and endogenous molecules (F.Y. Li et al., 2016; Miners et al., 2017).

In this study, we investigated the activities of four hepatic detoxication enzymes (EROD, BFCOD, GST and UGT) in fish exposed to lead (II) and paraquat, respectively. Different dose and time-dependent patterns of these enzymes in liver were characterized under lead (II) or paraquat exposure with different concentrations and experimental periods. Furthermore, the discrepant response of every enzyme in fish exposed to lead and paraquat was compared under the parallel conditions to investigate the toxic effects of the two pollutants.

2. Materials and methods

2.1. Chemicals and reagents

7-ethoxyresorufin, resorufin, 7-hydroxy-4-(trifluoromethyl)-coumarin, nicotinamide adenine dinucleotide phosphate (NADPH), uridine 5′-diphosphoglucuronic acid trisodium salt (UDPGA-3Na), reduced glutathione (GSH) and p-nitrophenol (p-Np) were obtained from Sigma-Aldrich Chemical Co. (USA). 7-benzyloxy-4-trifluoromethyl-coumarin (BFC) was purchased from Cypex (Dundee, Scotland, UK). Coomassie brilliant blue G-250 (CBG) was purchased from BBI (Toronto, Canada). Dithiothreitol (DTT) and phenylmethanesulfonyl fluoride (PMSF) were obtained from Amresco (USA). The ELISA was purchased from Nanjing Jiancheng Bioengineering Institute (China). All other chemicals were purchased from commercial Chemicals suppliers in Shandong, China.

2.2. Animals

The same batches of goldfish (*Carassius auratus*) with similar weight (5.9 ± 0.6 g) and length (5.2 ± 0.8 cm) were purchased from a local commercial fish farm (Jinan, China). Before the exposure experiments, all fish were adapted to laboratory conditions for 2 weeks in municipal water which were continuous aerated and dechlorinated. In the course of acclimation periods, fish were fed every other day with OSI freshwater granule food (Baolilai Feed Co. Ltd., Beijing, China). No food was offered during the whole exposure phase to reduce the external interference. The water quality of acclimation was monitored daily and continuously maintained at an adaptable condition for goldfish (temperature 20 ± 1 °C, pH 7.0 ± 0.2, and DO 6.5 ± 0.2 mg/L). The subsequent exposure water quality was in accordance with the condition of acclimation periods. Feces of fish and residual food were removed in time.

2.3. Exposure and sampling

To get a comparable result, the same series of exposure concentrations were designed with various exposure levels for lead and paraquat. Referring to the information of previous studies (Zhang et al., 2013; Huang et al., 2014; Ma et al., 2014; Nellore and Nandita, 2015; Chuntib and Jakmunee, 2015) and the preliminary experiments, 6 concentrations were eventually designated for the following toxicological experiments. Randomly selected 12 fish per group were kept in glass tanks (40 × 23 × 25 cm^3) with the specific concentrations of 0.05, 0.1, 0.5, 1, 5 and 10 mg/L for lead exposure. The same exposure experiments were conducted for paraquat groups besides supplementing 0.01 mg/L for the assay of GST activity. A shared blank control was performed simultaneously. Three replicate experiments were operated for every treatment and the control. All test groups and the controls were kept in