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Comparative proteomic analysis of ovary for Chinese rare minnow (*Gobiocypris rarus*) exposed to chlorophenol chemicals

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ABSTRACT

Pentachlorophenol (PCP) and 2,4,6-trichlorophenol (TCP) are suspected of disrupting the endocrine system and thus affecting human and wildlife reproduction, but the potential common mechanisms and biomarkers of chlorophenols (CPs) in the ovary are not fully elucidated. In the present study, the female rare minnow (*Gobiocypris rarus*) was exposed to PCP (0.5, 5.0, and 50 μg/L), TCP (1.0, 10, and 100 μg/L) and 17β-estradiol (as a positive control) for 28 days, and the matrix-assisted laser desorption/ionization (MALDI) tandem time-of-flight (TOF/TOF) mass spectrometry analysis was employed to investigate the alteration of protein expression in the ovary. After comparison of the protein profiles from treated and control groups, 22 protein spots were observed to be altered in abundance (>2-fold) from female treated groups, and 14 protein spots were identified successfully. These proteins were related to molecular response patterns, endocrine effects, metabolic pathways, and even the possible carcinogens in response to CP exposure. The seven differentially expressed mRNA encoding proteins were measured by quantitative real-time PCR (QRT-PCR) and histopathology was also measured. Our data demonstrate that alterations of multiple pathways may be associated with the toxic effects of CPs on ovaries.

Biological significance

Although numerous studies have shown the affection of the endocrine system with exposure to chlorophenols (CPs), there is little report on the alterations of protein expression in the ovaries from rare minnows following exposure to PCP or TCP. In the present study, a comparative

Abbreviations: ADSL, adenylosuccinate lyase; CPs, chlorophenol chemicals; DCP, 2,4-dichlorophenol; 2-DE, two-dimensional gel electrophoresis; EDCs, endocrine disrupting chemicals; DMSO, dimethyl sulfoxide; E-2, 17β-estradiol; H&E, haematoxylin and eosin; ER-β, oestrogen receptor; Lec-3, galectin-3; MALDI, matrix-assisted laser desorption/ionisation; PCP, pentachlorophenol; PIMT, L-isoaspartate O-methyltransferase; PMF, peptide mass fingerprint; QRT-PCR, quantitative real-time PCR; PI, protease inhibitor cocktail; SUMO, small ubiquitin-related modifier; TCP, 2,4,6-trichlorophenol; TOF/MS, tandem time-of-flight mass spectrometry; Vtg, vitellogenin.

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proteomic approach using two dimensional gel electrophoresis and mass spectrometry (MALDI-TOF/TOF MS) has been developed to identify certain proteins in the ovaries of Chinese rare minnow, whose abundance changes during exposure to CPs.

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1. Introduction

Chlorophenols (CPs) are toxic environmental pollutants that are ubiquitous in the aquatic environment due to their usage or improper disposal as a pesticide and wood preservative. Recent studies have found that CPs have hepatoxicity, and they are suspected of disrupting the endocrine system, subsequently affecting human and wildlife reproduction and even leading to cancer [1]. Because of their toxicity and adverse effects on humans and wildlife, the US EPA classified PCP and TCP as priority pollutants [2]. Some countries have banned or controlled the use of PCP [3], but other countries still use PCP to prevent fungal attacks on wood [4]. PCP was used in China during the 1970s to control schistosomiasis [5], and large concentrations of PCP (up to 103.7 μg/L) were detected in Dongting Lake [6]. PCP was banned in China as a pesticide in 1997 [7]; however, PCP is still used as a wood preservative [6]. Concentrations of PCP as great as 0.59 μg/L, 2,4-dichlorophenol (DCP) as great as 20.0 μg/L and TCP as great as 29.0 μg/L were observed in the surface water of seven major watersheds and three drainage areas of China [4,8].

Exposure to PCP affects the endocrine system of vertebrates and may lead to dysfunction of the immune system and disruption of normal sexual, cognitive, physical and emotional development [9,10]. PCP can cause endocrine disruption by either direct interaction with receptors or alteration of enzymes involved in steroid hormone synthesis and metabolism [11]. The mechanisms of endocrine disruption caused by PCP have been studied in vitro and in vivo, and the results indicate that PCP and TCP may inhibit steroidogenesis by disrupting cAMP signalling in a human adrenocortical carcinoma cell line (H295R) [12] and other oestrogenic activities, such as induction of vitellogenin (Vtg), in the cultured hepatocytes of male channel catfish [13]. In fish, oestrogenic activities (induction of Vtg) and reproductive impairment have been reported in the rare minnow [14], whereas alteration in serum testosterone has been observed with crucian carp exposure to PCP [15]. However, previous studies have mainly focused on single oestrogenic activity with respect to PCP, and there are few reports about the effect on global protein profiles in ovaries exposed to CPs. Thus, global protein functional analyses to explore the complicated endocrine-disruption mechanism of CPs are warranted.

Proteomics is a powerful method in toxicology and provides insight into the mechanisms of toxic compounds [16,17]. Specifically, the comparative proteomic approach is the main strategy of proteomics for analysing and comparing the differentially expressed proteins in toxicological studies combining two-dimensional gel electrophoresis (2-DE) and MALDI TOF/TOF analysis [18,19]. This method was used to study the mechanism of liver toxicity following PCP and TCP exposure in rare minnows [20,21], but an investigation of the protein expression profile in ovaries exposed to CPs has not yet been conducted. As an ideal model animal for toxicological studies, the Chinese rare minnow (*Gobiocypris rarus*) has been widely used for aquatic toxicity testing [14,20]. The fish is small (30–80 mm in length) and easy to culture in the laboratory. With its relatively short life cycle and spawning of hundreds of eggs with high fertilisation and hatching rates, the rare minnow is a suitable organism for aquatic toxicological tests of CPs [22].

With the aim of understanding the mechanisms of toxicity and disruption of the endocrine system caused by CP exposure, in the present study, rare minnows were treated with PCP and TCP for 28 days. Then, 2-DE combined with MALDI TOF/TOF approach was employed to examine the alterations in protein expression in the ovaries from rare minnows following this exposure to CPs. The identified protein response to CP exposure provides useful insights into ovary molecular response patterns, endocrine effects, metabolic pathways, cell functions most affected, and even the possible signalling pathway that disrupts reproduction and carcinogenicity in the ovaries of rare minnows. To further confirm the ovary proteins that are differentially expressed in response to the intake of CPs, the gene expression changes were monitored using quantitative real-time PCR (QRT-PCR) to complement the proteomic data. Based on the proteomic analysis together with the seven differentially expressed mRNA and histopathological results, these findings contribute to elucidating the complicated mechanism of CP toxicity in ovaries.

2. Materials and methods

2.1. Chemicals

Trichlorophenol (TCP: CAS No. 88-06-2), pentachlorophenol (PCP: CAS No 131-52-2), and 17β-estradiol (E-2: CAS No. 50-28-2) were all purchased from Sigma (USA). PCP, TCP and