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The detoxification responses, damage effects and bioaccumulation in the scallop *Chlamys farreri* exposed to single and mixtures of benzo[*a*]pyrene and chrysene

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ABSTRACT

This study aimed to investigate the detoxification responses, damage effects and biotransformation in scallop *Chlamys farreri* exposed to benzo[*a*]pyrene (BaP) (0.1, 1 μg/L), chrysene (CHR) (0.1, 1 μg/L) and BaP + CHR (0.1 + 0.1, 1 + 1 μg/L) for 15 days. Results demonstrated that BaP and CHR concentration (BaP < CHR) in tissues increased rapidly in a time and dose effect. The mRNA expression of aryl hydrocarbon receptor (AhR), cytochrome P450 1A1 (CYP1A1), CYP1B1, multidrug resistance protein 1 (MRP1/ABCC1), breast cancer resistance protein (BCRP/ABCG2) and P-glycoprotein (P-gp) were induced especially in the mixtures of BaP and CHR. Heat shock protein 90 (HSP90) and aryl hydrocarbon receptor nuclear translocator (ARNT) mRNA expression was significantly elevated at days 1, 10 and 15. Detoxification enzymes of 7-ethoxyresorufin O-deethylase (EROD), uridine-diphosphate-glucuronyl-transferase (UGT) and sulfotransferase (SULT) were significantly induced and then became stable gradually while glutathione-S-transferase (GST) was inhibited in the mixtures of BaP and CHR at days 10 and 15. Superoxide dismutase (SOD), glutathione peroxidase (GPx), catalase (CAT) and glutathione (GSH) were all stimulated especially in the mixtures of BaP and CHR. The levels of DNA strand breaks, lipid peroxidation (LPO) and protein carbonyl (PC) contents showed damage effects exposed BaP and CHR. All the results indicated that BaP and CHR have similar induced effect and a majority of the biomarkers pointed to a more toxic effect when BaP and CHR were mixed. These will provide a solid foundation for the study of PAHs detoxification mechanism in bivalves and valuable information for marine pollution monitoring.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs), with two or more fused aromatic rings, are mainly derived from petrogenic pollution, anthropogenic combustion, petroleum spillage and industrial and urban runoff (Sahu et al., 2009; Lüchmann et al., 2014), and even PAHs can be generated from the direct toasting (Rey-Salgueiro et al., 2008). Due to their persistent, bio-accumulative, toxic and long-range transportable properties, PAHs have drawn wide attention (Wania and Mackay, 1993; Pickering, 1999; Cachot et al., 2006; Yang et al., 2010). In 2008, the sum of benzo[*a*]anthracene (BaA), benzo[*a*]pyrene (BaP), benzo[*b*]fluoranthene (BbF) and chrysene (CHR) was identified as the most suitable indicator for PAHs in food (EFSA, 2008). According to previous studies, BaP and CHR contents in surface sediment from Zhanjiang Bay, China, were up to 19.90 and 39.65 ng/g dry weight, correspondingly (Huang et al., 2012). Total PAH concentrations ranged from 139.16 to 1717.87 ng/L in surface water, from 226.57 to 1404.85 ng/L dry weight in suspended particulate matter, and from 276.26 to 1606.89 ng/g dry weight in sediments, in the Bohai Sea, China (Men et al., 2009). Dissolved and particulate PAHs were up to 217 and 1597 ng/L in Northwestern Mediterranean Sea (Guigue et al., 2011). All above studies suggested that PAHs pollution had been a serious problem all over the world. Considering unpredictable interactions of environmental contaminants, studies on the toxic effect and risk assessment of single and mixtures of PAHs are urgently needed.

As one of the largest classes of chemicals present in the environment (Van der Oost et al., 2003) PAHs have been considered a health risk for wild life and humans for a long time (IARC, 1973; Ariese et al., 2001). Presently, growing attention has been concentrated on PAH metabolic mechanism in the organism, especially on their toxicity and metabolites (Barhoumi et al., 2011; Cavalieri and Rogan, 1995; Harris et al., 2009; Jacques et al., 2010; Ramesh et al., 2001). The detoxification responses of PAHs are a complex process that involves AhR pathway, phase I (EROD et al), phase II (GST, UGT and SULT et al) xenobiotic-metabolizing enzymes and phase III transporters (Paetzold et al., 2009; Pereira et al., 2011; Liu et al., 2014a, 2014b; Cai et al., 2016). CYP1 family plays an important role in metabolism of many toxicants including PAHs (Ghanayem et al., 2000; Matsumoto et al., 2007; Jönsson

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et al., 2010) with the catalytic activity of EROD. High induction of CYP1 transcripts by BaP was observed in rare minnow (Yuan et al., 2013). Moreover, Willett et al. (2006) reported that BaP exposure significantly induced CYP1B gene transcription in blood, gonad and liver of laboratory catfish. However, there was limited information on the expression of CYP1B1 mRNA in bivalve exposed to PAHs. The coordinated regulation of xenobiotic-metabolizing enzymes with xenobiotic transporters was also regarded as a mechanism of detoxification (Bard, 2000; Leslie et al., 2005). In mammals, arsenic (Liu et al., 2001), arsenite (Kauffmann et al., 2002) and BbF (Ebert et al., 2005) induced the mRNA expression levels of MRP1. Paetzold et al. (2009) reported that hepatic mRNA expression levels of MRP1 and BCRP were elevated in Tar Pond killifish in the side with higher PAHs level. Though the important role of EROD, GST, UGT and SULT were frequently studied in bivalves in PAHs metabolism, however, studies on the exclusion of metabolites were limited, especially the role of MRP1 and BCRP in scallop *C. farreri*.

PAHs could stimulate antioxidant defensive system and lead to oxidative damage through production of ROS during the process of detoxification in aquatic organisms (Livingstone et al., 1990; Akcha et al., 2000a; Pan et al., 2009; Bouraoui et al., 2009; Xiu et al., 2014). However, there were still few studies concentrating on the differences between BaP and CHR, as well as the relationship among detoxification system, antioxidant defensive system, damage effects and bioaccumulation induced by PAHs. It is well known that combining contaminants may lead to synergistic or antagonistic effects, which were different from individual contaminant (Groten, 2000; Celander, 2011). Oliveira et al. (2015) reported that the mixtures of BaP, dichlorodiphenyltrichloroethane (DDT) and tributyltin were more toxic to fish *Rhamdia quelen* than the single. However, there existed an antagonistic effect between BaP and DDT on green mussel *Perna viridis* (Song et al., 2016). Although aquatic animals had oxidative stress when exposed to BaP or CHR (Xiu et al., 2014; Ren et al., 2014; Ren et al., 2015), the toxic differences of BaP, CHR and their mixtures on scallop *C. farreri* were not well known.

Scallop *C. farreri* is an important commercial shellfish and is widely cultured in the shallow seas of China (Wang et al., 2007). It is also recommended as an indicator in toxicology studies (Pan et al., 2008; Liu, 2009) and environmental monitoring programs (Liu et al., 2012). Furthermore digestive gland is the major site of xenobiotic metabolism and biotransformation enzymes in molluscs (Livingstone et al., 1990, Livingstone, 1991; Hu et al., 2015a). In the present study, we investigated potential toxic effects of BaP, CHR and their mixtures in the digestive gland of scallop *C. farreri*, including the following endpoints: (a) the expression levels of detoxification-related genes (AhR, HSP90, ARNT, CYP1A1, CYP1B1, MRP1, BCRP, P-gp), (b) the activities of detoxification enzymes (EROD, GST, UGT, SULT), (c) the effect of antioxidant defense system (SOD, CAT, GPx, GSH), (d) levels of biomolecule damage parameters (*F* value, LPO levels and PC contents), (e) the bioaccumulation of BaP and CHR in digestive gland and soft tissue. The aim of present study is to offer the preliminary information on the metabolic pathway, damage effect and bioaccumulation of the single and mixtures of BaP and CHR in scallop *C. farreri*, and provide valuable information for the risk assessments of multiple chemicals.

2. Materials and methods

2.1. Chemicals

BaP (CAS#50-32-8) and CHR (CAS#218-01-9) were purchased from Sigma-Aldrich Chemical Co. (St. Louis, MO, USA). The chemicals for chemical analysis pretreatment and High Performance Liquid Chromatography (HPLC) detection were of chromatogram grade, obtained from E. Merck (Darmstadt, Germany). In addition, all the chemicals for toxic effects analysis were of analytical grade.

2.2. Experimental design

Healthy adult individuals of scallop *C. farreri* (6.06 ± 0.7 cm in shell length of) were obtained from the Shazikou shellfish farm (Yellow Sea, Qingdao, China). All the scallops were acclimated to laboratory condition in aquarium (1 L water per scallop) for seven days in filtered and aerated seawater pumped from Taiping bay before the exposure test. The salinity (30.7 ± 1.1), temperature (19.7 ± 1.0 °C) and pH (8.1 ± 0.8) were measured [average ± standard deviation (SD)] throughout the experimental periods and following the water exchange. The scallops were fed daily with dried powder of *Spirulina platensis* (30 mg/day for each individual).

The background concentration of BaP (0.153 ng/L) and CHR (0.139 ng/L) in seawater from Taiping bay was analyzed by HPLC before the experiment. Dimethyl sulfoxide (DMSO) was used as the vehicle of BaP and CHR, and the final concentration of DMSO was 0.001% in all tanks. And the DMSO test had been done in a preliminary experiment with the result that there was no obvious influence of DMSO on scallops. Scallops were divided randomly into seven groups, each of which consisted of three replicate aquarium (n = 100 scallops/aquarium) with 100 L of seawater. One group was used as control (seawater) and each of the remaining six groups was exposed to BaP: 0.1 μg/L (BaP0.1), 1 μg/L (BaP1) and CHR: 0.1 μg/L (CHR0.1), 1 μg/L (CHR1) isolated and in combination (mixtures of BaP0.1 + CHR0.1 or BaP1 + CHR1) for 15 days. The exposure concentrations were based on the concentration of PAHs in the coastal seawater in China, and in order to elicit clearly distinguishable effects, we set two relatively higher concentrations than the environmental concentration (Jin et al., 2014; Xiu et al., 2014; Wang et al., 2015). During the experimental period, half of the water was renewed every day, and the concentrations of BaP and CHR were reestablished.

Scallops were sampled (three replicates per treatment) at 0, 1, 3, 6, 10 and 15 days. Each replicate consisted of fifteen scallops, nine of which were used for BaP and CHR concentration analysis and six of which were used for the measurement of molecular biomarker. The day 0 control samples were selected randomly from each experimental aquarium for no difference between all groups before exposing to contaminants. The digestive gland and soft tissue of scallops were dissected and frozen immediately at −80 °C for subsequent examination.

2.3. Chemical analysis

The analysis of BaP and CHR in tissue was conducted in accordance with the standard method procedures (USEPA, 1996) with some modifications. Freeze-dried scallop tissue sample was Soxhlet extracted with acetone/dichloromethane mixture (1:1) at 70 °C for at least 18 h, and triplicate extracts for each of the scallop tissues were prepared. All extracts were dried using anhydrous sodium sulfate and gently evaporated to 2 mL by a rotary evaporator. The extraction aliquot was added into solid phase extraction column (3 ml PSA, CNW, China) after the column was preliminarily eluted with 5 mL hexane. Then 10 mL mixtures of dichloromethane and hexane (2:3) were eluted thrice to obtain the combined eluate. The eluate was dried with anhydrous sodium sulfate, concentrated by a rotary evaporator, reconstituted with 3 mL acetonitrile and finally filtered through a nylon syringe filter (0.22 μm, 13 mm, ANPEP, China).

The BaP and CHR quantification were analyzed with a high performance liquid chromatograph (HPLC, Shimadzu, Japan) equipped with two pumps, diode array detector and a reverse-phase C18 column (ZORBAX Eclipse PAH, 4.6 × 250 mm, 5 μm). The elution temperature was maintained at 34 °C and flow rate was 1 mL/min throughout. The initial mobile phase was 50% acetonitrile for 20 min, followed by 20 min linear gradient to 100% acetonitrile and keeping 100% acetonitrile for 15 min, then decreased to the initial phase in 10 min. 10 μL of concentrated extract were injected for each run. The detection limits for BaP and CHR were 0.01 μg/g d.w. Retention times were defined by

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