Basal and benzo[a]pyrene-induced expression profile of phase I and II enzymes and ABC transporter mRNA in the early life stage of Chinese rare minnows (Gobiocypris rarus)

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Abstract

ATP-binding cassette (ABC) transporters together with phase I and II detoxification enzymes have been considered as included in a cellular detoxification system. Previous studies have highlighted the involvement of aryl hydrocarbon receptor (AHR) and Cyp1a in PAH-induced embryo toxicity. However, the response of other xenobiotic enzymes/transporters in PAH-mediated embryo toxicity is not fully characterized. In the present study, rare minnow embryos were exposed to 10 and 100 μg/L BaP within 4 h post-fertilization (hpf) up to 168 hpf. RNA was extracted at 24, 48, 96, and 168 hpf. The basal and BaP-induced expression of phase I enzyme genes (cyp1a, 1b1, and 1c1), phase II enzyme gene (gstm and ugt1a), and ABC transporter genes (abc1b1, abc1c1, abc2b2, and abcg2) mRNA was determined using real-time PCR. Severe developmental defects (e.g., spinal deformities, pericardial and yolk-sac edema) were observed in the BaP treated groups. The basal expression showed that gstm was most strongly expressed, followed by abc1b1, ugt1a, and abc2c2, whereas cyp1a, 1b1, 1c1, and abcg2 showed weak expression. BaP significantly induced the mRNA expression of three CYP1s (cyp1a, 1b1, and 1c1) (p<0.05) and the ABC transporters (abc1c1, abc2c2, and abcg2) in a dose-dependent manner. However, the mRNA expression of Phase II enzymes (gstm and ugt1a) for the BaP treatments showed no significant difference with that of the controls. Furthermore, distinct induced patterns of these genes were observed during different exposure periods. Simultaneous up-regulation of the cyp and ABC transporter gene transcripts suggests that a possible involvement and cooperation in the detoxification process could provide protection against the BaP toxicity of rare minnows at the early life stage.

1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are ubiquitous organic contaminants arising from incomplete combustion or pyrolysis of organic material (Hendon et al., 2008). Early life stages of fish are especially sensitive to environmental pollutants, e.g., PAHs (Incardona et al., 2011). In previous studies, the developmental toxicity of PAHs in fish was well established (Incardona et al., 2004, 2006, 2011; Wassenberg and Di Giulio, 2004). PAH exposure produces a common syndrome of developmental defects in fish that include cardiovascular dysfunction, pericardial and yolk-sac edema, and craniofacial and spinal deformities (Incardona et al., 2005). In addition, these syndromes resemble the developmental defects caused by persistent organochlorine contaminants, such as 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) (Scott et al., 2011). The developmental toxicity of dioxin-like compounds is similar among vertebrate species, including mammals, birds, and fish (Jonsson et al., 2007a), and the mechanism is AHR-mediated and Cyp1a independent (Antkiewicz et al., 2006).

PAHs have been associated with developmental toxicity in the early life stages of many species by the ability of PAHs to bind to the aryl hydrocarbon receptor (AHR) (Billiard et al., 2006; Scott et al., 2011), a ligand-activated transcription factor that controls the expression of a battery of genes (e.g., cyp1a) involved in xenobiotic metabolism. However, PAH-induced developmental toxicity in fish is differentially dependent on AHR and Cyp1a metabolism (Incardona et al., 2005, 2006, 2011; Scott et al., 2011). Generally, the tricyclic PAHs (e.g., fluorene and phenanthrene) cause developmental abnormalities in an AHR-and Cyp1a-
independent manner, most likely by targeting cardiac ion channels (Incardona et al., 2005). However, retene (alkylphenanthrene)-induced developmental toxicity is AHR-mediated and Cyp1a independent, similarly to TCDD (Scott et al., 2011). In contrast, the developmental toxicity of some tetracyclic PAHs (e.g., pyrene and benz[a]anthracene) and pentacyclic PAHs (e.g., BaP) requires a functional AHR, but the tetracyclic compound chrysene produces no discernible developmental defects in fish (Incardona et al., 2006, 2011; Wassenberg and Di Giulio, 2004). Furthermore, dioxin-like compounds are poor substrates for Cyp, whereas some PAHs could be metabolized by Cyps and produce reactive metabolite intermediates and oxidative stress, which may contribute to the developmental toxicity in fish (Bauder et al., 2005; Timme-Laragy et al., 2009). Previous studies have highlighted the involvement of AHR and Cyp1a in PAH-mediated embryo toxicity (Incardona et al., 2011; Scott et al., 2011). However, relatively less studies have determined the expression properties and characterized the role of other xenobiotic enzymes (e.g., Cyp1c) during the early life stages of fish exposed to environmental pollutants (Jonsson et al., 2007a).

ATP-binding cassette (ABC) transporters together with phase I and II detoxification enzymes have been considered as a cellular detoxification system. ABC transporters are highly conserved transmembrane active transport proteins (Dean and Annilo, 2005), and several members of this transporter family confer multixenobiotic resistance (MXR) in aquatic organisms (Costa et al., 2011; Long et al., 2011). Previous studies using various animal models have indicated that P-glycoprotein (Abcb1), multidrug resistance-associated proteins 1–5 (Abcc1–5), and the breast cancer resistance protein (Abcg2) are the most relevant proteins associated with resistance to toxic substances (Leslie et al., 2005). Several studies have evaluated the effects of ABC transporters in fish exposed to environmental pollutants (Christine Paetzold et al., 2009; Diaz de Cerio et al., 2012). Phase I (e.g., Cyp450) and II (e.g., Gst) detoxification enzymes catalyze the reactions of xenobiotic metabolism, and the induction of phase I and II enzymes by environmental pollutants (e.g., PAHs) has been widely studied (Kopecka-Pilarczyk and Correia, 2009; Lu et al., 2009; Yeager et al., 2009). Previous studies indicated that ABC transporters could be regulated in a coordinated manner with phase I and II enzymes (Bard, 2000; Leslie et al., 2005; Xu et al., 2005). Recently, several studies have reported the simultaneous expression of biotransformation enzymes and ABC transporters in adult killifish, red mullet, and Nile tilapia (Christine Paetzold et al., 2009; Costa et al., 2011; Della Torre et al., 2010). Costa et al. (2012) evaluated the basal transcription patterns of ABC transporters, cyp1a, and gsta during the developmental stage of Nile tilapia. However, the full characterization of this detoxification system is still scarce, particularly for early life stage fish exposed to environmental pollutants (e.g., PAHs).

Benzo[a]pyrene (BaP) is one of the most studied environmentally relevant PAHs, and its carcinogenic and mutagenic properties have been extensively studied (Nicol et al., 1995; Tsukatani et al., 2003). In previous studies, BaP significantly induced the expression of cyp, gst, and ABC transporter genes in fish (Costa et al., 2011). In addition, several studies have reported BaP-mediated developmental toxicity in fish (Huang et al., 2011; Incardona et al., 2011; Matson et al., 2008). BaP is most often identified with cancer because BaP is a well-established chemical mutagen (Thompson et al., 2010), and BaP was used as a model chemical in this study. The Chinese rare minnow (Gobiocypris rarus) is considered an appropriate species for assessing endocrine disrupting chemicals due to its small size, ease of culture, short life cycle, and prolific egg production with high fertilization and hatching rates (Zha et al., 2008; Li et al., 2009; Yang et al., 2010). This minnow is distributed in the upstream region of the Yangtze River and in the Sichuan Province of China.

The transcription level is a major factor influencing the role of xenobiotic enzymes in substrate oxidation and effects in vivo (Jonsson et al., 2007b). Therefore, the aim of this study was to evaluate the basal and BaP-induced transcription patterns of the genes involved in fish detoxification (cyp1a, cyp1b1, cyp1c1, gstm, ugt1a, abc1, abc1c, abc2c, and abc2g) during the developmental stage of rare minnows to discern their role in embryo toxicity.

2. Materials and methods

2.1. Chemicals and test fish

Benzo[a]pyrene (purity > 98%) and acetone were purchased from Sigma (Chemical Co., USA). Stock solution of BaP was prepared by dilution in acetone.

The rare minnow brood stock was raised in a flow-through system with dechlorinated tap water (pH 7.2–7.6; hardness 44.0–61.0 mg CaCO3/L; and temperature 25 ± 1 °C) with a photoperiod of 16:8 h (light:dark), this stock has been used to test chemicals in our laboratory for more than 10 years (Li et al., 2009; Zha et al., 2008). Embryos in the experiment were obtained from the same pair of parent fish, following previous methods of our laboratory (Zha et al., 2008). All the fish or embryos used in the study were treated according to the International Guiding Principles for Biomedical Research Involving Animals (Council for International Organizations of Medical Sciences, http://www.cioms.ch) and approved by the institutions animal care and use committee of the Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences.

2.2. Experimental details

2.2.1. Exposure and developmental toxicity test

Fertilized eggs were collected within 4 h post-fertilization (hpf), pooled, and placed into dechlorinated tap water. A total of 540 rare minnow embryos were collected, subsequently, the embryos were randomly distributed into 3 exposure groups (vehicle, nominal water concentrations of BaP at 10, and 100 μg/L), each treatment group contained 3 replicate cylindrical glass containers (diameter, 9 cm; depth, 6 cm), and each replicate cylindrical glass containers included 60 embryos, a total of 180 embryos were used for each individual treatment. The exposure water was renewed every 24 h. During the experiment, the water temperature was maintained at 25 ± 1 °C, the dissolved oxygen was maintained at 8.0 ± 0.35 mg/L, and the pH was maintained at 7.0 ± 0.2. A vehicle treatment containing diluted acetone served as a control in the present study, the ratio of vehicle to water was 1:10,000 (v/v).

The embryos were observed every 24 h, and the number of embryos with yolk sac/pericardial edema and spinal deformities was counted. For observations, embryos were removed from the glass containers, scored in their surrounding water, and returned to the test system within 5 min. The detailed method for embryo toxicity test was according to previous studies (Zha et al., 2008; Hendon et al., 2008). Hatchability and time to hatch were calculated according to Zha's study (Zha et al., 2008). Specially, the deformity rate was scored at 96 hpf, calculating by using the number of the deformity fry (fry with yolk sac or pericardial edema or spinal deformities) relative to the number of all the remaining fry at 96 hpf. Digital images were taken at 96 hpf for all remaining fry using a Nikon Coolpix 4500 digital camera (Japan). The fish were anesthetized with ~1 mM MS-222 (Sigma, USA) for imaging when necessary.

2.2.2. BaP determination in exposure water

To determine the real concentration of BaP in the water of the exposure containers, both control (acetone) and BaP-treated water were sampled three times during the exposure period for actual BaP determination. Water samples were collected within 20 min after dosing and analyzed to confirm BaP concentrations from each exposure container. The detailed protocol for sample extracting and BaP concentrations analysis in the exposure water were according to previous study from our laboratory (Qiao et al., 2008). BaP was not detected in any of the acetone treated group. The actual concentrations of BaP in the water samples were 8.17 ± 0.52 and 5.73 ± 0.56 μg/L for the nominal concentrations of 10 and 100 μg/L BaP, respectively. When referring to the concentrations of BaP, nominal concentrations of BaP were used throughout the manuscript.

2.2.3. Sample, total RNA isolation and reverse transcription

10 embryos or 5 larvae were sampled from each replicate cylinder container and served as the sources for total RNA extraction at 24, 48 hpf (embryo stage) and 96, 168 hpf (larval stage), respectively. A total of 30 embryos or 15 larvae were sampled for each individual treatment per sampling time. Total RNA was extracted by the SV Total RNA Isolation System and following the manufacturer’s protocol (Promega, USA). The quantity and quality of the RNA were determined by a spectrophotometer (Thermo Fisher, USA), the ratio of OD 260/280 was range from
1.8 to 2.0. Then, RNA samples were dissolved in ribonuclease-free water and stored at −80 °C until the reverse-transcriptase polymerase chain reaction was performed.

The protocol of reverse-transcriptase polymerase chain reaction was described in a previous report from our laboratory (Li et al., 2009). The PCR reaction mixtures (25 μL) consisted of Brilliant II SYBR Green QPCR master mix, 300 nM forward primer, and 300 nM reverse primer. The primer pairs used for real-time PCR are shown in Table 1. For each sample, the gene expression was analyzed in triplicate using the following protocol: 95 °C for 8 min and 40 cycles of 30 s at 95 °C, 30 s at 57 °C and 30 s at 72 °C. Melting curve analysis was performed on the PCR products at the end of each PCR run to ensure that a single product was amplified.

2.2.4. Determining the gene expression by real-time PCR

To determine the basal and BaP-induced expression patterns of xenobiotic enzymes/transporters in the developing stages of the rare minnow, real-time PCR was performed in an Mx3005P real-time qPCR system (Stratagene, USA) according to previous study from our laboratory (Li et al., 2009). The PCR reaction mixtures (25 μL) consisted of Brilliant II SYBR Green QPCR master mix, 300 nM forward primer, and 300 nM reverse primer. The primer pairs used for real-time PCR are shown in Table 1. For each sample, the gene expression was analyzed in triplicate using the following protocol: 95 °C for 8 min and 40 cycles of 30 s at 95 °C, 30 s at 57 °C and 30 s at 72 °C. Melting curve analysis was performed on the PCR products at the end of each PCR run to ensure that a single product was amplified.

The basal mRNA expression of these genes was calculated by the 2−ΔΔCt method using p-actin as the reference gene, and the 2−ΔΔCt method was used for the fold-change in expression after exposure (Schmittgen and Livak, 2008). The mean value of these triplicate measurements was used for the calculations of mRNA expression, and p-actin was used for normalization.

2.3. Statistical analysis

Statistical analyses were performed using SPSS (version 13.0) and OriginPro (version 8.0). All quantitative data are expressed as the mean ± S.E. of the mean (SEM). The differences in the hatchability and mortality between the BaP-treated and control groups, and the transcript levels of these genes across different time points were evaluated using one-way ANOVA (p < 0.05), followed by Tukey’s test for multiple comparisons. The differences between the means of the BaP-treated and acetone-treated groups at the same time point were analyzed using Student’s t-test.

3. Results

3.1. Effects of BaP on developmental toxicity of rare minnow

At 24 and 48 hpf (embryo stage) and at 96 and 168 hpf (larval stage), the embryos/larvae were observed and scored for the presence/absence of yolk sac/pericardial edema, spinal deformities, hatchability, and mortality. Digital images were taken at 96 hpf for all remaining larvae (Fig. 1).

During the exposure period, significant differences in hatchability were observed among these treatment groups, however, no significant differences were observed in time to hatch compared with that of the control (p < 0.05) (Table 2). In addition, a significant increase in cumulative mortality was observed among the treatment groups, and 100% mortality was observed for the 100 μg/L BaP treatment before the end of exposure. Furthermore, the 10 and 100 μg/L BaP treatments showed severe developmental defects, such as yolk sac/pericardial edema and spinal deformities (Fig. 1), these defects were not present in the control.

3.2. Basal expression of xenobiotic enzymes/transporters during developmental stages of rare minnows

The basal expression levels of xenobiotic enzyme/transporter genes were compared at 24, 48, 96, and 168 hpf (Fig. 2). The basal expression represents the data from the acetone-treated groups (control), and all the data were presented as a percentage of the expression of each gene at 168 hpf (Fig. 2).

The transcription of all these genes was detected at 24 hpf, except for abcg2 (Fig. 2). During the experimental period, the expression levels of abcb1, gstm, ugt1a, and abcg2 were significantly increased (39.56-, 11.95-, 8.78-, and 7.8-fold relative to the expression of each gene at 24 hpf, respectively; p < 0.05), whereas the basal expression levels of cyp1a, cyp1b1, and abc2 were slightly elevated. Furthermore, abcb1, abc2, and abcg2 showed weak expression at the embryo stage (24 and 48 hpf), whereas their basal expression increased significantly at the larval stage (96 and 168 hpf) (Fig. 2).

The basal levels of these genes were also compared at 24 and 168 hpf (Fig. 3). These results showed that gstm was the most highly expressed, followed by abcb1, ugt1a and abc2. In contrast, cyp1a, cyp1b1, abc1, and abcg2 showed weak expression. For the three cyp genes, the expression level of cyp1c1 was much higher than those of cyp1a and 1b1 at 24 and 168 hpf, respectively (p < 0.05). For the ABC transporters, abcb1 and abc2 were highly expressed, whereas abc1 and abcg2 showed weak expression (Fig. 3).

3.3. Transcriptional effects of xenobiotic enzymes/transporters following BaP exposure

Total RNA was extracted at 24, 48, 96, and 168 hpf, and the transcriptional effects of xenobiotic enzymes and ABC transporter gene mRNA were determined using real-time PCR while rare minnow embryos were exposed to 10 and 100 μg/L BaP.

BaP significantly induced the expression of cyp1a, cyp1b1, and cyp1c1 relative to that in the same time point acetone-treated groups (Figs. 4 and 5, p < 0.05). Moreover, cyp1a was markedly more induced than the other two cyp1 genes. For cyp1a, the highest induction was observed at 24 hpf for the 10 and 100 μg/L BaP treatments (63- and 45-fold, respectively), relative to the same time point acetone-treated groups (Figs. 4 and 5). However, the level of cyp1a induction continuously decreased during the experimental period (Fig. 5). In contrast, cyp1b1 and cyp1c1 showed the highest induction at 48 hpf from the 100 μg/L BaP treatments (5.7- and 20-fold, respectively), relative to the same time point control (Figs. 4 and 5), and the induced pattern of these two genes
was similar over time during the exposure (Fig. 5). Furthermore, no significant changes in the gstm and ugt1a transcripts were observed for the BaP-treated groups \((p > 0.05)\) (data not shown). For the ABC transporters, the expression of abcc1, abcc2, and abcg2 for the BaP treatments was significantly elevated compared with the same time point acetone-treated groups \((Figs. 4 and 5, p < 0.05)\), whereas no significant change of abcb1 transcripts was observed in the BaP-treated groups \((p < 0.05,\) data not shown). The most inducible gene was abcg2. Moreover, the expression of abcc1 and abcc2 showed a time- and dose-dependent up-regulation \((Fig. 5)\). In contrast, the induced level of the abcg2 gene showed no significant changes during the exposure period \((Fig. 5)\).

### 4. Discussion

#### 4.1. Effects of BaP on developmental toxicity of rare minnows

The developmental toxicity of PAHs in fish has been well established in previous studies \(\text{(Incardona et al., 2004, 2006, 2011; Wassenberg and Di Giulio, 2004)}\). PAH exposure produces a common syndrome of developmental defects in fish that includes cardiovascular dysfunction, pericardial and yolk sac edema, and craniofacial and spinal deformities \(\text{(Incardona et al., 2005)}\). Our results on the BaP-induced developmental defects in rare minnows largely agree with previous studies \(\text{(Hendon et al., 2008)}\). Several studies have reported BaP-mediated developmental toxicity in fish \(\text{(Huang et al., 2011; Incardona et al., 2011; Matson et al., 2008)}\). BaP-mediated bradycardia and pericardial edema are AHR2 dependent in zebrafish embryos \(\text{(Incardona et al., 2011)}\). In addition, Cyp1a inhibition enhanced the developmental toxicity of BaP in killifish, and Cyp1a appears to play a protective role \(\text{(Wassenberg and Di Giulio, 2004)}\). Furthermore, oxidative stress...
may be a component of this developmental toxicity, and antioxidant responses could provide protection against PAH challenges during development (Bauder et al., 2005; Timme-Laragy et al., 2009).

4.2. Basal expression of xenobiotic enzymes/transporters during developmental stages of rare minnow

During the experimental period, the basal levels of \textit{abcb1}, \textit{gstm}, \textit{ugt1a}, and \textit{abcg2} were highly increased. At 24 and 168 hpf, \textit{gstm} was the most highly expressed, while \textit{cyp1a} showed weak expression (Fig. 3). Previously, Wu et al. (2011) reported an increase in oxidative stress during the embryogenesis of Japanese medaka. In addition, the early life stages of fish are highly sensitive to environmental stress; thus, the high basal expression of \textit{gstm} could result from the role of the Gsts in maintaining oxidative stress balance (Costa et al., 2012) and protecting against chemical injury (Garner and Di Giulio, 2012). Furthermore, the low basal expression of \textit{cyp1a} transcript in rare minnow embryos is similar to the previously obtained results (Jo\textsuperscript{nsson et al., 2007b}).

For three \textit{cyp} genes, the basal level of \textit{cyp1c1} expression was much higher than those of \textit{cyp1a} and \textit{1b1} (Fig. 3). Similar results showing higher expression for \textit{cyp1c1} relative to \textit{cyp1a} and \textit{1b1} were observed in zebrafish (Jonsson et al., 2007b) and killifish embryos (Wang et al., 2006). These results indicated that the \textit{cyp1c1} genes could play a role in fish embryo development (Jonsson et al., 2007b). For the ABC transporters, \textit{abcb1}, \textit{abcc2}, and \textit{abcg2} showed a weak expression at the embryo stage (24 and 48 hpf), whereas their basal expression significantly increased at the larval stage (96 and 168 hpf). Previous studies have reported an increased basal expression level of ABC transporters after the hatching period in several aquatic species (Costa et al., 2012; Faria et al., 2011). Hatching is a critical period during the development of fish, this means the loss of their protective “shell”, and the embryos will be directly exposed to the surrounding environment, and may require an increase in the endogenous defense mechanisms (e.g., increase expression of the ABC transporters) (Costa et al., 2012). This may partially explain the reason for the increased basal expression levels of ABC transporters after the hatching period observed in our study and previous studies (Costa et al., 2012; Faria et al., 2011).

4.3. Transcriptional effects of xenobiotic enzymes/transporters following BaP exposure

Acetone treatment (low concentration) had no effects on \textit{cyp} gene expression and showed no developmental toxicity in zebrafish compared with a water control (Jonsson et al., 2007a). Thus, a vehicle treatment containing diluted acetone served as a control in the present study, the ratio of vehicle to water was 1:10,000 (v/v).

In the present study, BaP significantly induced the expression of \textit{cyp1a}, \textit{1b1} and \textit{1c1} (relative to the same time point acetone treated group) during the early life stages of rare minnows. Previous studies have reported the elevated expression of \textit{cyp1a}, \textit{1b1} and \textit{1c1} caused by various environmental pollutants during the early life stages of fish (Jonsson et al., 2007b; Timme-Laragy et al., 2007). In addition, \textit{cyp1a} was markedly more inducible than the other two \textit{cyp} genes during the exposure period. This result largely agrees with previous studies in killifish and zebrafish (Jonsson et al., 2007b; Wang et al., 2006). 

Cyp1a inhibition
Fig. 3. Relative basal mRNA expression of xenobiotic enzymes/transporters of rare minnows compared at 24 and 168 hpf. The relative expression of each mRNA was calculated by the $2^{-\Delta Ct}$ method (where $\Delta Ct$ is the value obtained by subtracting $Ct$ of $\beta$-actin mRNA from $Ct$ of the target mRNA). $\beta$-actin was used for normalization. The data are presented as a percentage of the expression of $gstm$ at each time point. The basal expression represents the data from the acetone-treated groups (control). The data are shown as the means ± SEM. A significant difference between the groups of $p < 0.05$ ($n = 3$, ANOVA) is indicated by differences in the letters above the bars.

Fig. 4. Fold changes of the xenobiotic enzyme/transporter gene expressions between BaP-treated groups and the control in rare minnow embryos at each time point (only showed the gene expressions which significantly changed at each time point). The transcripts of $abcg2$ were not detected at 24 hpf, and 100% mortality was observed in 100 µg/L BaP treated groups before conclusion of exposure at 168 hpf. Calculations were made using $\beta$-actin as reference gene and the 24 hpf acetone group as a calibrator (the expression of $abcg2$ using 48 hpf acetone group as a calibrator). Data are shown as mean ± SEM. Asterisks (*) denotes significant differences between BaP treated groups and the control at each time point using Students $t$-test ($^*p < 0.05$, $^{**}p < 0.01$; $n = 3$).
enhanced the developmental toxicity of β-naphthoflavone in zebrafish (Billiard et al., 2006), and Cyp1a appears to provide a protective role. Thus, the relatively high induction of cyp1a could be to rapidly eliminate endogenous and exogenous AHR agonists (Jonsson et al., 2007a) and to provide protection against BaP toxicity in rare minnows. Moreover, the level of cyp1a induction continuously decreased over time during the exposure, in contrast, cyp1b1 and 1c1 showed different induced patterns from that of the cyp1a gene, and the induced patterns of these two genes were similar over time during the exposure. Consistent with this, a previous study showed that the tissue distribution patterns of cyp1b1 and cyp1c exhibited a marked similarity in adult zebrafish; furthermore, phylogenetic analyses indicated that the cyp1b1 and cyp1c genes are more closely related to each other than they are to the cyp1a (Jonsson et al., 2007b).

However, no significant changes in the gstm and ugt1a transcripts were observed in the BaP-treated groups during the developmental stages of rare minnows. In previous studies, the gstp2 gene was significantly up-regulated by BaP in zebrafish embryos (Garner and Di Giulio, 2012), and elevated expression of gstm was observed in adult killifish from a pond heavily polluted with PAHs and PCBs (Christine Paetzold et al., 2009). Olsvik et al. (2010) assessed the transcriptional responses of six gsr genes in early larvae of Atlantic cod exposed to dispersed oil, but only small effects on gsr transcription could be observed. In contrast to gsr genes, some ugt1 isoforms are classical AHR battery genes, and elevated expression of ugt1 by various environmental pollutants have been observed in fish (Jones et al., 2010). Moreover, a study in our laboratory showed significantly induced expression of ugt1a mRNA by BaP (6.9-fold relative to control) in adult rare minnows (unpublished). Moreover, a previous study in mice showed that Nrf2, in addition to AHR, is required for TCDD induction of the classical AHR battery genes nqo1, ugt1a6, and gsta1, as well as many other ugt and gsr isoforms (Yeager et al., 2009). Thus, the different induced patterns of the ugt1a gene may result from the difference in these two receptors between embryo and adult rare minnows.

Several members of the ABC transporter family (e.g., abcb1, abcc2, and abcg2) confer multixenobiotic resistance (MXR) in aquatic organisms. In this study, the expression of abcc1, abcc2, and abcg2 was significantly elevated for the BaP treatments compared with that of acetone-treated groups (p < 0.05). Previous studies also showed the elevated expression of ABC transporters in various fish species exposed to environmental pollutants (Christine Paetzold et al., 2009; Costa et al., 2011). Moreover, the most inducible gene in this study was abcg2. Abcg2 (BCRP) is a transporter of BaP conjugates that are metabolically formed in Caco-2 cells and induced by AHR agonists (Ebert et al., 2005), and Tan et al. (2010) confirmed the AHR dependence of abcg2 gene regulation in humans. Moreover, Abcg2 protects the developing fetus from possible toxins in the case of exposure via the placenta (Robey et al., 2009). Thus, the relatively high induction of abcg2 could be related to a possible role of the transporter in the efflux of BaP metabolites during the early life stages of rare minnows. The level of abcg2 transcripts did not significantly changed during the exposure period, whereas the expressions of abcc1 and abcc2 showed a time- and dose-dependent up-regulation. The different induced patterns suggest different regulated mechanisms between abcg2 and the abcc genes. In previous studies, abcc1 and abcc2 have been shown to act synergistically with several phase II
conjugating enzymes (Leslie et al., 2005), and abcc2 shares considerable similarities with abc1 in substrate specificity and structure characteristics (Long et al., 2011).

In the present study, BaP simultaneously up-regulated the expression of the cyp and ABC transporter genes at 48 and 96 hpf for both 10 and 100 μg/L BaP treated groups. Simultaneous up-regulation of biotransformation enzymes and ABC transporters by various environmental pollutants has been reported in adult fish (Christine Paetzold et al., 2009; Costa et al., 2011; Della Torre et al., 2010). In addition, previous studies indicated that the ABC transporters could be regulated in a coordinated manner with phase I and II enzymes (Bard, 2000; Leslie et al., 2005; Xu et al., 2005). Thus, the simultaneous up-regulation of the cyp and ABC transporter genes by BaP suggests a possible involvement and cooperation of these genes in the detoxification process during the developmental stage of rare minnows.

5. Conclusion

In summary, BaP exposure caused severe developmental defects, including spinal deformities, pericardial and yolk-sac edema, and high mortality in rare minnow embryos and larvae at exposure concentrations tested. Basal expression showed that gstm was most highly expressed, followed by abcb1, ugt1a1 and abcc2. In contrast, cyp1a, cyp1b1, abc1, and abcg2 showed weak expression. Furthermore, BaP significantly up-regulated three cyp1 genes as well as the ABC transporters (abc1, abcc2, and abcg2). Simultaneous up-regulation of the cyp and ABC transporter genes suggests a possible involvement and cooperation in the detoxification process and provides protection against BaP toxicity during the early life stage of rare minnows. However, further studies are required to discern the role of these genes in embryo toxicity.

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