Comparative Biochemistry and Physiology, Part C 196 (2017) 19–26

Contents lists available at ScienceDirect

Comparative Biochemistry and Physiology, Part C

journal homepage: www.elsevier.com/locate/cbpc

Developmental toxicity in flounder embryos exposed to crude oils derived from different geographical regions

Jee-Hyun Jung [a,b], Eun-Hee Lee [a], Kwang-Min Choi [a], Un Hyuk Yim [a,b], Sung Yong Ha [a], Joon Geon An [a], Moonkoo Kim [a,b,*]

[a] Oil and POPs Research Group, Korea Institute of Ocean Science and Technology, Geoje 53201, Republic of Korea
[b] Department of Marine Environmental Science, Korea University of Science and Technology, Daejeon 34113, Republic of Korea

ARTICLE INFO

Article history:
Received 7 December 2016
Received in revised form 1 March 2017
Accepted 2 March 2017
Available online 06 March 2017

Keywords:
Oil spill
PAHs
Embryo
Developmental toxicity
Geographical origin

ABSTRACT

Crude oils from distinct geographical regions have distinct chemical compositions, and, as a result, their toxicity may be different. However, developmental toxicity of crude oils derived from different geographical regions has not been extensively characterized. In this study, flounder embryos were separately exposed to effluents contaminated by three crude oils including: Basrah Light (BLO), Pyrenees (PCO), and Sakhalin Vityaz (SVO), in addition to a processed fuel oil (MFO-380), to measure developmental toxicity and for gene expressions. Each oil possessed a distinct chemical composition. Edema defect was highest in embryos exposed to PCO and MFO-380 that both have a greater fraction of three-ring PAHs (33% and 22%, respectively) compared to BLO and SVO. Observed caudal fin defects were higher in embryos exposed to SVO and MFO-380, which are both dominated by naphthalenes (81% and 52%, respectively). CYP1A gene expressions were also highest in embryos exposed to SVO and MFO-380. Higher incidence of cardiotoxicity and lower *nkx* 2.5 expression were detected in embryos exposed to PCO. Unique gene expression profiles were observed in embryos exposed to crude oils with distinct compositions. This study demonstrates that crude oils of different geographical origins with different compositional characteristics induce developmental toxicity to different degrees.

1. Introduction

Depending upon their geographic region of origin, crude oils have distinct chemical compositions, and, as a result, their toxicities may be different (Fingas, 2014; Incardona et al., 2004; Kim et al., 2012). While there are a plethora of studies describing adverse effects of crude oils on adult or juvenile/embryonic aquatic animals (Agamy, 2012; Brette et al., 2014; Dubansky et al., 2013; Dussauze et al., 2015; Esbaugh et al., 2016; Hicken et al., 2011; Holth et al., 2014; Huang et al., 2012; Kim et al., 2013a; Lockhart et al., 1996; Mager et al., 2014; Perkins et al., 2005; Zhang et al., 2012), there is a paucity of studies to identify the underlying mechanisms of developmental toxicity in embryonic fish exposed to crude oil. This is likely the case because crude oils contain a complex mixture of PAHs, making it difficult to determine which specific PAH is responsible for the toxicity. In previous studies, we characterized the developmental toxic effects on zebrafish, spotted seabass, and olive flounder exposed to Iranian Heavy crude oil (IHCO) from the *Hebei Spirit oil spill* (December 7, 2007), and compared them to the reported developmental toxicity caused by weathered Alaska North Slope crude oil (Jung et al., 2013; Jung et al., 2015a; Jung et al., 2015b). Despite some differences in the physical and chemical properties of these two crude oils derived from different geographic regions, their cardiotoxicities in developing zebrafish embryos were remarkably similar. This similarity was likely because both oils predominantly contained three- and four-ring PAHs. We also demonstrated that IHCO exposure resulted in disruption of developmental processes related to biotransformation, cardiac formation, and immune-defense system in embryonic olive flounder (*Paralichthys olivaceus*) using high-throughput RNA-sequence analyses (Jung et al., 2016). Some studies compared general toxicity (e.g., LC_{50}) of different types of crude oils and refinery products on the tropical/subtropical species, including fish (clownfish, *Amphiprion clarkia*; silverside minnows, *Menidia beryllina*; sheepshead minnow, *Cyprinodon variegatus*), mysid (*Mysidopsis almyra*), shrimp (penaeid shrimp, *Penaeus vannamei*; grass shrimp, *Palaemonetes pugio*; brown shrimp post-larvae, *Penaeus aztecus*), and sea urchin larvae (*Arbacia unctulata*, *Dendraster excentricus*) (Andersen et al., 1974; Neff et al., 2000). Incardona et al. (2013) also showed the comparative toxicity between weathered Alaska North Slope Oil (ANSCO) and Mississippi Canyon 252 (MC252) oil on fish embryo and larvae. Although they compared the toxicity of two different crude oils, the morphological defects and patterns of cytochrome P450 induction were largely indistinguishable and generally correlated with polycyclic aromatic compound (PAC)

* Corresponding author at: Oil and POPs Research Group, Korea Institute of Ocean Science and Technology, 41 Jangmok-1-gil, Geoje 53201, Republic of Korea.
E-mail address: mkim@kiost.ac.kr (M. Kim).

http://dx.doi.org/10.1016/j.cbpc.2017.03.003
1532-0456/

composition of each oil type. What remains unclear is whether crude oils from different geographical regions differentially induce developmental toxicities in marine embryonic fish. Systematic comparative studies of molecular and morphogenesis endpoints in embryonic marine fish exposed to geologically distinct crude oils are rare.

Many types of crude oil are produced around the world, including the Middle East, Asia-Pacific, Africa, Europe, and America (BP, 2016). Kim et al. (2012) reported that compositions of alkanes and PAHs were distinguishable among crude oils derived from different global regions, and that these differences may promote distinct developmental defects in embryonic fish. Comparative studies of toxic responses to crude oils derived from different geographical regions may facilitate the toxicological assessment of biological consequences induced by recent and future oil spills. Incardona et al. (2004) reported that significantly different effects and underlying toxic mechanisms were observed in zebrafish (*Danio rerio*) embryos exposed to seven individual non-alkylated PAHs, including naphthalene, fluorene, dibenzothiophene, phenanthrene, anthracene, pyrene, and chrysene. The aforementioned study demonstrated that different PAHs can induce different effects on fish during their early life stages.

To clarify the relationship between developmental toxicity and different crude oils having different PAH composition, we evaluated gross malformation and multiple molecular biomarkers including biotransformation pathways, immune response, cardiac function, and oogenesis in embryonic flounders. Flounder are demersal fish species found at the bottom of coastal areas around the world and also in estuaries. They are key species for artificial seedling production and juvenile release (Kim et al., 2007). They are winter spawning species and produce buoyant, pelagic eggs that develop in the coastal upper water column. Therefore, they are susceptible to the exposure to oil slick on the sea surface during oil spill incidents, which can be well simulated in the gravel effluent-continuous flow system (Incardona et al., 2005). In our previous study using the environmentally relevant exposure system (gravel effluent-continuous flow system), developmental defects were more severe in flounder embryos than those of spotted seabass, which is also pelagic and winter spawning species (Jung et al., 2015a). Consistent with the severity of defects, flounder embryos also accumulated higher tissue levels of PAHs. To our knowledge, this is the first comparative study aimed at comparing the developmental toxicity of distinct crude oils derived from different global regions in embryonic marine fish species using multiple biochemical and malformation markers.

2. Material and methods

2.1. Fish embryos and exposure

Artificially fertilized embryonic flounders (*Paralichthys olivaceus*) were purchased at a commercial fishery station (Ihwa-sangrok, Geoje, Korea). The fertilized eggs were transported to the laboratory and exposed in a continuous flow-through exposure system after acclimation in an exposure tank. Developing embryos (48 h post-fertilization (hpf) in optic vesicle stage) were incubated in fifteen 40 L exposure tanks [40 cm (width) × 45 cm (length) × 24 cm (height)]. Approximately 30,000 embryos in each tank were exposed to effluents from gravel coated by three crude oils in triplicates, including Basrah Light (BLO), Pyrenees (PCO), and Sakhalin Vityaz (SVO) derived from Iraq, Australia, and Russia, respectively, in addition to a processed fuel oil (MFO-380). The MFO-380 produced in Korea is a blend of bunker A and bunker C. It is processed to reduce sulfur and other pollutants to protect the environment and is one of the most shipped oil internationally. The thirteenth through fifteenth tanks served as no oil exposure controls. Gravel was manually coated with test oil (1.5 g oil/kg gravel) by shaking in an uncoated stainless steel can. Coated gravel was then air dried for 24 h to form a thin layer of oil and loaded into glass generator columns (Jung et al., 2015a, 2015b). Filtered seawater was continuously percolated (0.6 L h^{-1}) through the columns to produce oil-contaminated seawater for toxicity tests. Water quality variables (i.e., pH, salinity, and dissolved oxygen) were monitored daily. Incubation temperature was maintained at 16 °C, and triplicate embryo samples (~300 embryos per replicate) were randomly collected from each tank after 4, 8, 24, and 48 h of exposure. Column flow rates and temperature were monitored, and embryos were examined daily to remove abnormal or nonviable ones.

2.2. PAHs in oils

PAHs were measured in three crude oils (BLO, PCO, SVO) and a fuel oil (MFO). Approximately 0.02 g of oil was dissolved in 1.0 mL of hexane and spiked with 50 μL of surrogate standards (a mixture of deuterated naphthalene, acenaphthene, dibenzothiophene, phenanthrene, chrysene and perylene: 10 ppm each). Each oil solution was transferred to a 3 g activated silica gel column topped with anhydrous sodium sulfate (~1 cm), and eluted with 30 mL of hexane/dichloromethane (50/50). The eluents were concentrated to 1 mL of hexane, and, then, a GC internal standard (terphenyl-d14) was added before instrumental analysis. Sixteen PAHs, prioritized by USEPA, and selected alkyl-substituted PAHs were analyzed using a gas chromatograph (Hewlett-Packard HP6890) coupled with a mass spectrometer (Hewlett-Packard HP5972). Total PAHs (ΣPAHs) is the sum of C0–C4 naphthalenes, acenaphthylene, acenaphthene, C0–C3 fluorenes, C0–C4 phenanthrenes, anthracene, fluoranthene, pyrene, benz[*a*]anthracene, C0–C3 chrysenes, benzo[*b*]fluoranthene, benzo[*k*]fluoranthene, benzo[*a*]pyrene, indeno[1,2,3-*cd*]pyrene, dibenz[*a*,*h*]anthracene, benzo[*ghi*]perylene, and C0–C3 dibenzothiophenes. Detailed gas chromatographic conditions, mass spectrometric settings, and quantification method were previously described in Yim et al. (2005).

2.3. Embryo imaging

Following 48 h of exposure, flounder embryos were observed and imaged using a Nikon SMZ800 stereomicroscope or a Zeiss Axioplan 2 compound microscope as previously described (Incardona et al., 2004, 2013; Jung et al., 2013). Pericardial edema and dorsal curvature, and other distinct defects in affected embryos were clearly distinguishable from unaffected ones (Supplementary Fig. 1). Frequency percentage was measured for pericardial edema, caudal finfold defect, and dorsal body axis curvature delay.

2.4. Quantitative comparison of mRNAs among groups

Total RNA was extracted from embryos (30 mg) using Isogen (Wako, Japan). The purified total RNA was reverse transcribed into cDNA using a first-strand cDNA synthesis kit (Invitrogen, USA). Quantitative PCR was performed using two-step procedures. β-Actin gene was used as a positive control for RT-PCR. Ratio of optical density at 260 nm and 280 nm (OD260/280) was approximately 1.9, and OD260/230 was approximately between 1.8 and 1.9. PCR primers and PCR conditions are shown in Table 1. To quantify mRNA expression level, comparative CT method ($2^{-\Delta\Delta Ct}$ method) was used in Roto-Gene Q (Qiagen, Germany) according to the manufacturer's instruction. All experiments were performed in triplicate (Jung et al., 2015b).

3. Results

3.1. PAH compositions of crude oils

In all oils measured, concentrations of alkylated PAHs far exceeded unsubstituted parent PAHs. Although the same amount of oil was applied to coat the gravel, the relative compositions of PAHs varied among oils, reflecting their different geographical origins (Table 2). In MFO, two-ring PAHs (C0- to C4-naphthalenes) were the most abundant and accounted for 52.2% of the total PAHs,