Embryonic exposure to PFOS induces immunosuppression in the fish larvae of marine medaka

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A B S T R A C T

Perfluorooctane sulfonate (PFOS) is a global pollutant that has been studied because of its health risks. PFOS has been shown to have immune toxicity. However, few studies have focused on the immune responses of fish larvae exposed to PFOS at early embryonic stages. In this study, the larvae of marine medaka (Oryzias melastigma) were evaluated for postnatal immune toxicity after embryonic exposure to PFOS (0, 1, 4 and 16 mg/L) from 2 days post fertilization (dpf). The physiological indices, survival rates, PFOS elimination kinetics, liver histology and gene transcription in the fish larvae were examined after depuration. The elimination rate constant (ke) of PFOS in the fish larvae ranged from 0.04 ± 0.00 to 0.07 ± 0.01 d⁻¹. Embryonic exposure to PFOS severely compromised the postnatal survival of fish larvae after depuration. The survival rate and body width decreased in a concentration dependent manner. PFOS impaired the liver structure in the fish larvae by enlarging the cell nuclei and damaging the cell structure. To explore the toxic mechanisms that affect the immune responses, fish larvae at 27 days post hatch (dph) were exposed to lipopolysaccharides (LPS) to elicit an inflammatory response. The inflammatory response and immune-related genes were generally up-regulated in the fish larvae following embryonic exposure to 0 mg/L PFOS. In contrast, the genes were all markedly down-regulated in the fish larvae following embryonic exposure to 1 and 4 mg/L PFOS. These results suggest that early life exposure to PFOS could alter immunoregulation functions, leading to functional dysfunction or weakness of the immune system in fish larvae. The immunosuppression effects caused by PFOS could reduce the efficiency of immune defense mechanisms and increase the susceptibility to infectious agents, which may contribute to various detrimental health effects in the fish larvae.

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1. Introduction

Perfluorooctane sulfonate (PFOS) has been widely acknowledged as a man-made persistent organic pollutant due to its low rate of degradation, global distribution, high levels of bioaccumulation and various toxicological effects (Giesy and Kannan, 2001; Lau et al., 2007). It has been used for a wide range of industrial purposes and consumer applications, including industrial surfactants, emulsifiers, fabrics, carpets, shampoo, food packaging, nonstick cookware, insecticides, fire-fighting foam, and other household products (Jeon et al., 2010).

Abbreviations: CAT, Catalase; COX-1, Cyclooxygenase-1; COX-2, Cyclooxygenase-2; dpf, Days post fertilization; dph, Days post hatch; DMSO, Dimethyl sulfoxide; GPX, Glutathione peroxidase; IL-8, Interleukin-8; IL-1, Interleukin-1 beta; LPS, Lipopolysaccharides; PFOS, Perfluorooctane sulfonate; PPAR, Peroxisome proliferator-activated receptor; qRT-PCR, Real-time quantitative reverse transcriptase polymerase chain reaction; SOD, Superoxide dismutase; TNF-α, Tumor necrosis factor-alpha; UCP2, Uncoupling protein 2.

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4 mg/L and 16 mg/L) accumulated in the embryos of marine medaka, which resulted in fragile fish with markedly decreased survival rates within one week, especially after embryonic exposure to 16 mg/L PFOS (Fang et al., 2012; Wu et al., 2012). Several other animal studies in rats and fish have reported that maternal exposure to PFOS can cause a variety of negative health effects on the F1 offspring, including deficits in birth weight, body weight, and skeletal abnormalities. However, few studies have focused on immunosuppression in the offspring of organisms following early exposure to PFOS, in particular those that have been challenged using LPS during the recovery period.

There is evidence to support mediation of immunosuppression by inflammatory cells and inflammation-associated products, including cytokines, chemokines, and reactive oxygen/nitrogen species (Ben-Baruch, 2006). However, few studies have investigated the effects of PFOS on immune toxicity in gene expression profiles related to above factors, particularly in marine organisms.

In this study, fish larvae were recovered in clean seawater following embryonic exposure to four doses of PFOS (0 mg/L, 1 mg/L, 4 mg/L, and 16 mg/L). The 0 mg/L exposure treatment was defined as the control. These exposure doses selected within the range of experimental concentrations commonly used by numerous toxicology studies (0.1–25 mg/L) and may represent some extreme conditions (Shi et al., 2008; Lin et al., 2009; Huang et al., 2010; Fang et al., 2012). The physiological indices, survival rates, PFOS elimination kinetics and liver histology of the fish larvae were examined. Escherichia coli (E. coli) and Pseudomonas aeruginosa (P. aeruginosa) were utilized to induce an immune reaction in the fish larvae to assess the immune toxicity effects of PFOS. The expression profiles of 12 genes related to the inflammatory response were investigated, including TNF-α, IL-1β, interleukin-8 (IL-8), uncoupling protein-2 (UCP2), glutathione peroxidase (GPX), catalase (CAT), cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), peroxisome proliferator-activated receptor α (PPARα), β (PPARβ, γ), and γ. These were investigated by real-time quantitative reverse transcription polymerase chain reaction (RT-PCR).

2. Materials and methods

2.1. Experimental procedure

PFOS (98 percent pure) was purchased from Tokyo Chemical Industry, Co. Ltd., Tokyo, Japan and dissolved in dimethyl sulfoxide (DMSO) to prepare the working solution. The embryo exposure experiment was designed based on previous studies (Fang et al., 2012; Huang et al., 2011). Briefly, a total of four PFOS exposure groups (0.1 mg/L, 1 mg/L, 4 mg/L, and 16 mg/L) with three replicates were set up. Meanwhile, a total of 100 fertilized embryos were exposed to each replicate group from 2 dpf until hatching. At 12 dpf (0 dpf), the hatched fish larvae from each treatment group were collected for depuration. The hatching rates of the embryos at this stage ranged from 71.2 percent to 93.4 percent, and each depuration group contained 70 fish larvae. The fish larvae were transferred to 250 mL polypropylene beakers containing 200 mL clean artificial seawater without PFOS and the salinity was controlled at 30%. The seawater was renewed daily. A total of four depuration groups with three replicates were set up.

The experimental process during the depuration period was designed as follows: The PFOS body burdens were detected at 0 dpf, 7 dpf and 17 dpf to determine the PFOS elimination rate constant in the fish larvae. At 17 dpf, the survival rates, growth parameters and liver histology of the fish larvae were examined. At 27 dpf, the fish larvae exposed to 1 and 4 mg/L PFOS were exposed to E. coli LPS for 12 h to quantify the expression profiles of the inflammatory response and immune-related genes. This developmental stage in teleost fish represents the morphological and functional maturity of the immune system and the presence of B-cells, which are essential components of the adaptive immune system and critical factors in responding to the immune toxicity of PFOS (Keil et al., 2008; Lam et al., 2004; Seppola et al., 2009). The experimental process is shown as follows:
Three live fish larvae from each replicate of each depuration group were randomly collected and homogenized using a pestle (Omega, USA) in 200 μL deionized water. Liquid chromatography (LC) (Shimadzu Prominance LC-20A, Japan) in conjunction with tandem mass spectrometry (MS/MS) (Applied Biosystems 3200Q TRAP, USA) was used to determine the PFOS body burdens in Oryzias melastigma larvae. The sample extraction procedure was performed according to our previous study (Fang et al., 2012). Briefly, 10 ng C13 PFOS was added to each sample (Welllington Laboratories Inc., Canada) as an internal standard. Methylenebuthyl ether (MTBE) was used to extract PFOS three times. All of the extracts from the organic layer were combined and evaporated under a gentle stream of nitrogen before reconstitution in 1 mL methanol, which was vortexed for 30 s and transferred into an auto sampler vial.

Each white phase contained MQ water (A) and methanol (B), and the flow rate was maintained at 500 μL/min. The gradient was increased from 5 percent to 90 percent B after 5 min and maintained for 2.5 min. It was increased to 100 percent B for 2.5 min and returned to the original conditions. A 5 μL sample was injected into a kinete, 2.6 μl C4, 100 A column (100 mm × 4.6 mm, 2.5 μm particle size, Phenomenex, USA). The temperature of the column was 40 °C. The multiple reaction monitoring (MRM) mode was operated in the MS/MS procedure, and the mass transition monitored ions were selected as follows: 503 → 99 for C13 PFOS, 499 → 99/80 for PFOS. Quantification of PFOS in samples was performed using a seven point external calibration curve consisting of 1 mL methanol and spiked with the PFOS in the concentration range 0–400 ng/mL. The standard curves of PFOS were linear and the correlation coefficient was 0.9998. The extraction recovery percentage of PFOS was measured using 3 blank fish larvae with 200 μL deionized water spiked with 3 known amounts of PFOS (2.5 μg/g, 25 μg/g and 100 μg/g wet weight) and 10 ng C13 PFOS. Each sample contained three independent replicates and extracted using the same procedure as calibration standards and samples. The concentrations of PFOS were calculated from standard curves, and the mean extraction recovery of PFOS through the entire analytical procedure ranged from 81 percent to 105 percent. The detection limit of the method for PFOS was 0.172 μg/L.

2.3. Detection of survival rates and growth parameters of the larvae at 17 dph

The survival rates of the fish larvae from each replicate in each depuration group were calculated at 17 dph. A total of five fish larvae of similar sizes from each replicate in each depuration group were collected and weighed on an electronic balance, and the average body weight of each larva was calculated by dividing the total weight by five. The fish larvae were anesthetized with MS 222 (Sigma, USA) and the body lengths and widths were measured using two-dimensional measuring software (CF-2000C, Changfang Optical Instrument Co. Ltd. Shanghai, China PR) under a stereo microscope (XTL-340, Changfang Optical Instrument Co. Ltd. Shanghai, China PR) mounted with a CCD camera (CF-2098, Changfang Optical Instrument Co. Ltd. Shanghai, China PR). Twelve fish larvae were randomly sampled for body length and width measured from each depuration group.

2.4. Effects of PFOS on liver histology in fish larvae at 17 dph

For liver histological examination, four fish larvae sampled from each depuration group at 17 dph were dissected by removing the fins, tail, and skull roof. The body cavity was opened to allow penetration of the digestive tract and the body cavity was opened to allow penetration of the intestinal cavity. The gut and the liver were removed and were fixed in Zenker’s fluid for 24 h at room temperature. The fixed samples were subsequently dehydrated in ethanol at gradient concentrations (30% for 10 min, 70% for 10 min, 100% for 10 min) and then embedded in paraffin wax with the profile upwards and sectioned at 5 μm. Serial sagittal sections were cut on a rotary microtome (Leica RM2125, Germany). The sections were mounted on a glass microscope slide and stained with hematoxylin and eosin (H & E) and observed under a Zeiss light microscope (Axio imager A1, Germany). The sizes of the liver and hepatocyte nuclei were evaluated by measuring the area using an image analysis system (WinROOF ver 6.4, Mitani Corp., Tokyo, Japan). Twenty cell nuclei areas were randomly selected for area measurement from each liver slide. The aqeous exposure of E. coli LPS (serotypes O55:BS, purchased from Sigma, Deisenhofen, Germany) was performed according to previous experiments conducted in zebrasfish larvae (Novoa et al., 2009). Three marine medaka larvae were sampled from each replicate, in each group at 12 h and immediately transferred into liquid nitrogen and stored at –80 °C until RNA preparation. The fish larvae were homogenized with a glass homogenize device in 1 mL RNA-Solv reagent (Omega Bio-Tek, Inc. Norcross, USA). Total RNA was extracted from the homogenates using a total RNA kit II (Omega Bio-Tek, Inc. Norcross, USA) according to the manufacturer’s instructions. After RNA extraction, reverse transcription (RT) was performed using the PrimeScript RT master mix perfect real time kit (DRRO36A, Takara) in a thermal cycler (Eppendorf, Germany) as follows: 37 °C for 15 min, 85 °C for 5 s, and maintained at 4 °C. The cDNA were diluted 1:5 for the second-strand PCR reaction. qRT-PCR was conducted using a SYBR Premix Ex TaqTM kit (Takara) in a Roche Light Cycler 480 II according to the manufacturer’s instructions. Primer sequences of the genes for qRT-PCR were designed according to our previous studies (Fang et al., 2012; Huang et al., 2012) and are presented in Table 1. A standard curve for each pair of real-time RT-PCR primers was constructed by running a set of 10-fold serial dilutions of the cDNA (group with LPS exposure, but without PFOS exposure). The amplification efficiencies for all the primers were 0.94–0.97 (R2 = 0.999). The PCR thermal profiles were performed as follows: an initial denaturation step at 95 °C for 30 s, followed by 40 cycles of 95 °C for 5 s, 60 °C for 34 s, and dissociation curve analysis. The real-time RT-PCR analyses contained 3 replicates from each group, and three PCR repeats were performed for each replicate. Eighteen second RNA expression levels were selected to normalize the gene expression levels, which were stable among the same tissue samples, in different tissues, and stages of development in fish and were unaffected by endocrine disrupting chemicals exposure. The relative fold changes in the gene expression are calculated based on the 2−ΔΔCt method (Livak and Schmittgen, 2001).

2.6. Data analysis

Data were tested for normality, homogeneity of variance and normal distribution before statistical analysis. Statistical analysis was performed using the SPSS 16.0 for Windows software package (SPSS Inc.). One-way ANOVA was performed to examine the differences between the experimental groups, followed by Tukey’s test (post hoc test). Differences were significant at p < 0.05.

3. Results

3.1. PFOS body burden in fish larvae from 0 dph to 17 dph

Changes in the PFOS body burden in fish larvae are shown in Fig. 1. The PFOS body burden gradually decreased from 0 dph to 7 dph during the depuration period, ranging from 8.3 ± 2.5, 20.6 ± 1.0 μg/g, 55.9 ± 12.8 μg/g wet weight to 3.3 ± 0.9, 20.9 ± 5.5 μg/g, 40.6 ± 1.7 μg/g wet weight after embryonic exposure to 1 mg/L, 4 mg/L and 16 mg/L PFOS, respectively. The PFOS levels in the O. melastigma larvae decreased during the subsequent depuration period from 7 dph to 17 dph, attaining similar levels of 1.0 ± 0.1 μg/g, 1.1 ± 0.1 μg/g, 1.3 ± 0.6 μg/g wet weight after embryonic exposure to 1 mg/L, 4 mg/L and 16 mg/L PFOS, respectively, at 17 dph. The PFOS body burdens were significantly lower (p < 0.05) than those from the same experimental groups at 0 dph. The elimination rate constant (ke) of PFOS by O. melastigma larvae were calculated according to the formula described in Ankley et al. (2004). The ke of PFOS ranged from 0.04 ± 0.00 d−1 to 0.07 ± 0.01 d−1 after embryonic exposure to 1 mg/L, 4 mg/L, and 16 mg/L PFOS from 0 dph to 17 dph during the depuration period.

3.2. Survival rates and growth parameters of fish larvae at 17 dph

The survival rates of the fish larvae 17 dph after embryonic exposure to 0 mg/L, 1 mg/L, 4 mg/L and 16 mg/L PFOS are shown in Fig. 2. The survival rates of the fish larvae were significantly decreased (p < 0.05) in a concentration dependent manner. At the higher doses of PFOS (4 mg/L and 16 mg/L), the survival rates of the fish larvae decreased to 28.3% and 16.6% respectively, at 17 dph. The PFOS body burdens were significantly lower (p < 0.05) after embryonic exposure to the highest dose (16 mg/L) or three doses (1 mg/L, 4 mg/L and 16 mg/
L) of PFOS at 17 dph. However, the body weights of the hatched larvae did not change significantly (p > 0.05) after embryonic exposure to all of the doses (1 mg/L, 4 mg/L and 16 mg/L) of PFOS during the same period.

### 3.3. Effects of PFOS on the liver histology of sh larvae at 17 dph

We examined the paraffin sections of the livers of the fish larvae 17 dph after embryonic exposure to four doses of PFOS (0 mg/L, 1 mg/L, 4 mg/L, 16 mg/L) using light microscopy (Fig. 3A–D). From the quantitative digital images analyses for the ratio of cell nuclei areas, it was found that the cell nuclei of liver did not exhibit any obvious histopathological changes between the control and exposure groups at 1 mg/L and 4 mg/L PFOS. However, the fish larvae after embryonic exposure to 16 mg/L PFOS showed marked enlargement of the cell nuclei compared with the control group (Fig. 3E).

### 3.4. The effects of LPS on gene expression in fish larvae at 27 dph

The transcriptional responses of several inflammatory and immune-related genes in fish larvae at 27 dph are shown in Fig. 4. In the control group, the expression of two key pro-inflammatory cytokines (TNF-α and IL-1β) was comparable (p > 0.05) following LPS exposure, but the pro-inflammatory cytokine IL-8 was significantly up-regulated (p < 0.05). Other selected genes, including SOD, CAT, COX-1, COX-2, UCP2, PPARα, PPARβ and PPARγ, were all significantly up-regulated (p < 0.05) after LPS exposure for 12 h compared with the fish larvae that were not exposed to LPS. The expression of GPX did not change significantly (p > 0.05). In contrast, all of the selected genes except for PPARγ in the fish larvae following embryonic exposure to 1 and 4 mg/L PFOS were significantly down-regulated (p < 0.05) compared with the control groups that were not exposed to LPS.

### Table 1

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequences used for qRT-PCR (5′ to 3′)</th>
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<tr>
<td>TNF-α</td>
<td>F: TGGTGTCCTTCTGACCCGGACTCAAGCTGCTCA</td>
</tr>
<tr>
<td></td>
<td>R: ACCTGTCGCGACTAAAGGATGTC</td>
</tr>
<tr>
<td>IL-1β</td>
<td>F: AGGCCGAGCAGCCCCAAAGTCTCA</td>
</tr>
<tr>
<td>IL-8</td>
<td>F: TGGTGTCCTTCTGACCCGGACTCAAGCTGCTCA</td>
</tr>
<tr>
<td></td>
<td>R: ACCTGTCGCGACTAAAGGATGTC</td>
</tr>
<tr>
<td>SOD</td>
<td>F: TGGGTACATATTTCAGCTGTCCTCA</td>
</tr>
<tr>
<td>GPX</td>
<td>F: AGGCACGCGACAGCCGACTCAAGCTGCTCA</td>
</tr>
<tr>
<td></td>
<td>R: ACCTGTCGCGACTAAAGGATGTC</td>
</tr>
<tr>
<td>COX-1</td>
<td>F: AGGCCGAGCAGCCCCAAAGTCTCA</td>
</tr>
<tr>
<td></td>
<td>R: ACCTGTCGCGACTAAAGGATGTC</td>
</tr>
<tr>
<td>COX-2</td>
<td>F: ACTGTCGACGCCGAGCTGACGATCA</td>
</tr>
<tr>
<td></td>
<td>R: TTACAGCACCACAGAACCCTCCTCT</td>
</tr>
<tr>
<td>PPARα</td>
<td>F: AAAAAAGATGGCTTCTGGTGCTT</td>
</tr>
<tr>
<td>PPARβ</td>
<td>F: ATGGAGTTGAAGCCCTGTCGGAGA</td>
</tr>
<tr>
<td>PPARγ</td>
<td>F: AGCCCTCTCGGTCTCCAGCTGCTAA</td>
</tr>
<tr>
<td>18 s (control)</td>
<td>F: GCCATGCTCGGGAGAACCAAGCTCCT</td>
</tr>
<tr>
<td></td>
<td>R: TGGTGTCCTTCTGACCCGGACTCAAGCTGCTCA</td>
</tr>
</tbody>
</table>

*: F means forward primer.

**: R means reverse primer.

### 4. Discussion and conclusion

In our previous and present studies, we found that exposure to PFOS (1 mg/L, 4 mg/L, and 16 mg/L) at an early developmental stage severely compromises the postnatal survival of marine medaka fish larvae and causes deformation and decreased body lengths and widths in the surviving larvae (Fang et al., 2012; Wu et al., 2012). However, the underlying mechanisms that influence the toxicological effects of PFOS remain largely unknown. It is widely acknowledged that there are strong relationships between biokinetics and the toxicological effects of contaminants (Wang et al., 2010). Therefore, the elimination of PFOS in fish larvae is a basic requirement for evaluating the toxic mechanisms of this compound. In this study, the body burden of PFOS decreased significantly during the depuration period, particularly after 7 dph. Until 17 dph, the PFOS body burdens in the fish larvae were lower than the levels in some fish livers from Tokyo bay, Flanders and an accidental spill site (1.8–72.9 μg/g) (Moody et al., 2002; Taniyasu et al., 2003; Hoff et al., 2005). The elimination rate constant (k e) of PFOS in the larvae of marine medaka ranged from 0.04 d −1 to 0.07 d −1, which is slightly higher than the rate in the whole body or different tissue organs in rainbow trout (Oncorhyncus mykiss) and the northern leopard frog (Rana pipiens) (k e=0.02–0.05 d −1) following dietary and aqueous exposure to PFOS (Ankley et al., 2004; Brandsma et al., 2011; Martin et al., 2003a, b). This discrepancy is partly because different species have different capacities for eliminating PFOS and may be attributed to different levels of salinity in the exposure water, which can affect the toxicokinetics of PFOS in the organisms (Jeon et al., 2010) and should be a focus of future research.
mediators of the inflammatory response (Corsini et al., 2011; Mollenhauer et al., 2010). However, to our knowledge, studies regarding suppression of these pro-inflammatory cytokines at the gene expression level to explore the immunotoxicity of PFOS are limited. In this study, we found that IL-8 was significantly upregulated in the control group after LPS exposure, although TNF-α and IL-1β remained unchanged. This discrepancy may be because IL-8 is a more sensitive marker than other cytokines in response to injury and inflammation (Sun et al., 2005). The suppression of pro-inflammatory cytokine expression in fish larvae following embryonic exposure to PFOS after a LPS challenge suggests that immunosuppression due to PFOS occurs by altering the pro-inflammatory cytokine transcriptional response.

UCP2 is a mitochondrial inner membrane protein that has been shown to be a negative regulator of macrophage reactive oxygen species (ROS) production and the primary genes that encode antioxidant enzymes, including SOD, CAT, and GPX, play important roles in protecting organisms from oxidative damage (Arsenijevic et al., 2000; Liu et al., 2007). In this study, the up-regulation of UCP2 and the antioxidant enzymes encoded genes in the fish larvae after exposure to LPS in the control group potentially play important roles in protecting cells against cytokine-mediated toxicity and oxidative stress (Alves-Guerra et al., 2003; Lortz et al., 2000). However, these gene expression patterns were all down-regulated in the fish larvae after embryonic exposure to PFOS, indicating that PFOS could cause an imbalance in the redox
state, induce excessive oxidative stress and impair the antioxidant capacities in the fish larvae, which may trigger systemic diseases later in life (Roberts et al., 2006; Vaziri et al., 2003). Previous studies have reported that the reactive oxygen/nitrogen species produced by oxidative stress may play critical roles in mediating immunosuppression in neoplasias and that the oxidative stress accompanied by immunosuppression due to toxicant exposure was also observed in goldfish (Ben-Baruch, 2006; Fatima et al., 2007). In the future study, we should further study the oxidative stress effects of PFOS and the fish response using the various methods, including the determination of oxidative stress and lipid peroxidation levels in the fish larvae and find their interactions with the gene expression patterns.

Cyclooxygenase (COX)-1 and (COX)-2 are critical components of the inflammatory response and can be induced by several pro-inflammatory cytokines (Lacroix and Rivest, 1998; Smith et al., 1998). LPS stimulated the expression of COX isoforms in the fish larvae in the control group, which is associated with the induction of inducible nitric oxide synthase (iNOS) and can lead to inflammatory disorders and tumor promotion (Smith et al., 1998). After embryonic exposure to PFOS, the expression patterns of COX-1 and COX-2 in the fish larvae were suppressed, which may be associated with an anti-inflammatory reaction in the immune cells (Surh et al., 2001). The anti-inflammatory reaction may result in a compensatory increase in anti-inflammatory mediators, which could transform the immune response into a state of immunosuppression and influence the development of various diseases (Novotny et al., 2011).

Multiple lines of compelling evidence have indicated that the three peroxisome proliferator–activated receptors (PPARα, β and γ) are all involved in regulating many important physiological processes, including lipid metabolism, energy homeostasis, reproduction, and inflammatory responses (Wahl and Michalik, 2012). PFOS exposure elicits the transcriptional responses of PPAR

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**Fig. 4.** The expression of genes related to the inflammatory and immune responses at 27 dph. represents the fish larvae following embryonic exposure to 0 mg/L PFOS (control group) without exposure to LPS. represents the fish larvae following embryonic exposure to 0 mg/L, 1 mg/L and 4 mg/L PFOS with exposure to LPS. The relative expression level was measured by SYBR qRT-PCR. Eighteen second rRNA was used as an internal control. The data were run in triplicate and are expressed as the mean ± SD relative to the control. *p < 0.05, **p < 0.001 indicates the different groups exposed to LPS compared with the control group that was not exposed to LPS. *p < 0.05, **p < 0.001 indicates the different groups exposed to LPS compared with the control group exposed to LPS. n = 3 for each replicate; three replicates were conducted.
isofoms in fish, mice and humans, which can trigger various developmental, reproductive and immune toxicity effects (Dong et al., 2012; Fang et al., 2012; Takacs and Abbott, 2007). In this study, we found that the three PPAR subtypes were all up-regulated in the fish larvae from the control group compared with that without LPS exposure, indicating that the pro-inflammatory responses were stimulated by LPS and the activated expression of PPARs was likely associated with anti-inflammatory effects (Wahl and Michalik, 2012). In contrast, LPS repressed the three PPAR subtypes in the fish larvae after embryonic exposure to PFOS compared with the control groups with LPS exposure or not, which may reduce the potential anti-inflammatory roles of PPARs and contribute to an enhanced inflammation phenotype. Recent studies have revealed that chronic or prolonged inflammation could also lead to immunosuppression, which is the normal outcome of the body’s defense system to avoid excessive immune stimulation in the early phases of infection, but may reduce efficiency of the host immune defense mechanisms and increase the susceptibility to infectious agents in later developmental stages (Fatima et al., 2007; Julia et al., 2012). Early exposure to PFOS likely causes chronic or prolonged inflammation in the marine medaka, which could subsequently suppress the host immune response later in life.

The liver is a major target organ for PFOS accumulation and the dysregulation of inflammation-related gene transcription following exposure to environmental pollutants and is frequently accompanied by liver damage in fish species, including the accumulation of lipid droplets, disruption of hepatocyte membrane integrity, and impaired hepatic cell structure (Hoff et al., 2003). There are also connections between inflammatory reactions and development of liver cancer (Berasain et al., 2009). It was previously reported that liver damage and immunosuppression occurred simultaneously in transgenic (TG) mice after viral infection (Soguero et al., 2002). In this study, obvious histopathological changes in the liver were detected in the fish larvae after embryonic exposure to the highest dose of PFOS, which is in agreement with the symptoms observed in female Chinese rare minnows treated with the highest concentration of amitrole after a recovery period (Li et al., 2009). These results indicate that the liver damage caused by PFOS is a long-lasting effect, and the relationships between this pathological phenomenon and the immunotoxicity of PFOS needs to be further clarified.

In conclusion, PFOS that accumulated in the embryonic stages was eliminated from the fish larvae during the depuration period. However, a lower postnatal survival rate, developmental retardation, and hepatotoxicity were observed following embryonic exposure to PFOS. After LPS stimulation, the expression of immune-related genes was decreased following PFOS exposure compared with the fish larvae without PFOS exposure, indicating that early exposure to PFOS could cause functional dysfunction or weakness of the immune system in the fish larvae. The immunosuppression effects caused by PFOS could impair the efficiency of the immune defense mechanisms and increase the risk of infection, which may contribute to various detrimental health effects in the fish larvae.

Acknowledgments

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