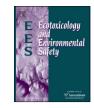
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# Embryonic exposure to PFOS induces immunosuppression in the fish larvae of marine medaka

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# ABSTRACT

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  $0.04 \pm 0.00$  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 downregulated 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). Although production and application of PFOS was phased out by the 3M Company at the end of 2002, it is still a global pollutant that is dispersed widely throughout the environment and has even been detected in remote polar areas (Armitage et al., 2009; Jogsten et al., 2012). Recent studies have reported that PFOS concentrations in the surface water of ocean, some coastal areas and river estuaries ranged from < 10 pg/L to 703 ng/L (Pan and You, 2010; Zhao et al., 2012). However, in the waste water and nearby river system of a semiconductor fabrication plant, these levels could reach up to 12,566 mg/L and 5.4 mg/L, respectively (Lin et al., 2009). In various edible fish muscles, the PFOS levels ranged from 0.27 ng/g to 5.98 ng/g wet weight (Zhao et al., 2011; Vestergren et al., 2012). In the livers, the maximum PFOS levels could reach up to  $1.8 \,\mu g/g = 9.03 \,\mu g/g$  and 72.9 µg/g in some fish species from Tokyo bay, Flanders and an accidental spill site (Moody et al., 2002; Taniyasu et al., 2003; Hoff et al., 2005).

Despite the widespread distribution of this compound, its toxicological and biologic effects, particularly the postnatal impacts on the offspring of organisms, are relatively unknown. In previous studies, we found that three doses of PFOS (1 mg/L,

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 $\beta$ , 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- $\alpha$ , 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 size and body weight. larval mortality, postnatal growth retardation, developmental delay, structural malformations and neurobehavioral defects (Du et al., 2009; Han and Fang, 2010; Ribes et al., 2010). In humans, PFOS can transfer to developing infants by crossing the placental barrier. It is present in the cord blood and can have adverse effects by reducing the birth weight, ponderal index and head circumference in infants, which may be closely related to the development of obesity, diabetes, and cardiovascular diseases later in life (Apelberg et al., 2007).

Although various apparent adverse effects have been observed in the offspring of organisms following prenatal exposure to PFOS, the underlying toxic mechanisms remain largely unknown. It is widely recognized that PFOS has estrogenic and endocrine disrupting activities (Du et al., 2009, Fang et al., 2012). The immune system serves as a potential target for endocrine disrupters, particularly environmental estrogens (Ansar Ahmed, 2000). Accumulating evidence has indicated that several endocrine disrupting chemicals could interfere with the transcription of genes related to the innate immune system during the early developmental stages of zebrafish (Danio rerio) and that the elevated estrogen levels could contribute to an attenuated anti-viral immune response, resulting in pregnancy-associated morbidities in mice (Jin et al., 2010; Pazos et al., 2012). There is a general consensus that exposure to PFOS alters the immune process in some experimental biological models (DeWitt et al., 2009). For example, natural killer (NK) cell functions and sheep red blood cell (SRBC)-specific immunoglobulin m (IgM) antibody synthesis were significantly decreased in male mice pups whose mothers were exposed to PFOS during pregnancy, which may impair the tumor and viral surveillance of the immune system and increase disease susceptibility in the pups (Keil et al., 2008). In particular, inflammation is one of the first responses of the immune system following exposure to xenobiotics, which may also be a significant factor involved in the development of many diseases (Barrett, 2012). There is a lot of evidence showing that the transcription of several genes related to the inflammatory response play profound roles in affecting the functional capacity of the immune system in offspring, including the pro-inflammatory cytokines interleukin-1 beta (IL-1 $\beta$ ) and tumor necrosis factor-alpha (TNF- $\alpha$ ), which were both elevated in the brains of rat pups following prenatal exposure to PFOS (Zeng et al., 2011).

The inflammatory response can be stimulated by bacterial lipopolysaccharides (LPS), which has been shown to be an effective approach in various animal models (Watzke et al., 2007). Bacterial LPS are the major constituents of the external laver of gramnegative bacteria, which are termed endotoxins, and can activate host innate immunity by stimulating phagocytic cells to produce pro-inflammatory cytokines (Swain et al., 2008). In many fish species, LPS often serves as protective agents against pathogens, and the inflammatory response in fish can be stimulated by injection of or immersion in bacterial LPS (Gonçalves et al., 2012; Novoa et al., 2009; Watzke et al., 2007). However, PFOS has been shown to have an inhibitory effect on the LPS-induced inflammatory response, cytokine secretion and humoral immune effects in mice and human immune cells (Corsini et al., 2011; Mollenhauer et al., 2010; Peden-Adams et al., 2008). The effects of immune toxicity caused by PFOS may give rise to immunosuppression in the test organisms, which can reduce the activation and efficacy of the immune system and lead to immunodeficiencies or immunocompromised responses that may result in an increased susceptibility to infection (Ben-Baruch, 2006). Few studies have focused on immunosuppression in the offspring of organisms following early exposure to PFOS, in particular organisms 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, prostaglandins 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.

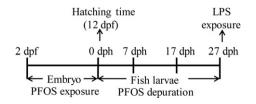
In this study, fish larvae were recovered in clean seawater following embryonic exposure to four doses of PFOS (0 mg/L, 1 mg/L, 4 mg/L, and 16 mg/L). The 0 mg/L exposure treatment was defined as the control. These exposure doses selected within the range of experimental concentrations commonly used by 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 LPS (serotypes 55:B5) 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 and immune functions, including TNF- $\alpha$ , IL-1 $\beta$ , interleukin-8 (IL-8), uncoupling protein 2 (UCP2), superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2), peroxisome proliferator-activated receptor  $\alpha$ ,  $\beta$ ,  $\gamma$  (PPAR $\alpha$ ,  $\beta$ ,  $\gamma$ ), were investigated by real-time quantitative reverse transcriptase polymerase chain reaction (gRT-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 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 dph), 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 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 dph, 7 dph and 17 dph to determine the PFOS elimination rate constant in the fish larvae. At 17 dph, the survival rates, growth parameters and liver histology of the fish larvae were examined. At 27 dph, 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:



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