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The *in vivo* action of chronic bisphenol F showing potential immune disturbance in juvenile common carp (*Cyprinus carpio*)

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HIGHLIGHTS

- BPF exposure increased ROS content and induced oxidative stress.
- BPF exposure altered the immune-related parameters.
- The mode of action of BPF is related to the NF-κB signaling pathway.
- The effects of BPF on the immune response are comparable to those of BPA.

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ABSTRACT

Bisphenol F (BPF) has been increasingly introduced into industrial applications as a replacement for bisphenol A (BPA), and has emerged as a ubiquitous environmental contaminant worldwide. Few studies have assessed the *in vivo* toxicities of BPF, particularly long-term exposure toxicities. In the present study, we examined whether long-term BPF exposure *in vivo* would evoke oxidative stress in the immune system of juvenile common carp. The results suggested that BPF exposure increased ROS content, oxidative stress indices, complement component 3, and immunoglobulin M contents, as well as the expression of inflammatory cytokine genes. Moreover, higher levels of *nf-κb* p65 gene expression were correlated with the induced ROS content and NF-κB pathway-associated genes, a strong indication that the mode of action of BPF is related to the NF-κB signaling pathway. We also provide evidence that the effects of BPF are comparable to those of BPA with regards to regulation of the immune response in teleosts, and therefore suggest that such chemical analogs should be thoroughly evaluated for their potential toxicity before they can be considered as "safer" replacements.

1. Introduction

Bisphenol F (BPF), a substitute for bisphenol A (BPA), is widely used in the manufacture of epoxy resins and polycarbonates (Usman and Ahmad, 2017). BPF contains two hydroxyphenyl groups that are structurally similar to those in BPA. BPF has a broad range of industrial applications, for example in the manufacture of lacquers, varnishes, liners, adhesives, plastics, water pipes, dental sealants, road and bridge deck toppings, tissue substitutes, and coatings for food packaging (Zheng et al., 2016). The broad usage of BPF has resulted in ubiquitous environmental pollution from such sources as contaminated dust, water, sediment, and sludge (Liao et al., 2012b; Yang et al., 2014b; Wang et al., 2015). Liao et al. (2012a) reported that concentrations of BPF in indoor dust in the United States were as high as 0.054 μg/g. BPF has also been reported to reach levels up to 1.74 μg/L in the Tamaraw River in Japan

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(Yamazaki et al., 2015), and 338 μg/kg (dry weight [dew]) in sediments (Liao et al., 2012b) and 384 μg/kg dw in sewage sludge (Lee et al., 2015) in Korea. As a result of its wide use in food packing, BPF has been detected in foodstuffs and soft drinks at levels up to 2.5 μg/kg fresh weight and 0.36 μg/L, respectively (Liao and Kannan, 2014; Yang et al., 2014c). Moreover, in south China, BPF has been detected in human urine at levels of 0.214 ng/mL (Yang et al., 2014a). In addition, several studies have reported that the degradation rate of BPF is similar to that of BPA (Rochester and Bolden, 2015). As such, it is believed that the increasing production of BPF and its subsequent growing presence in the environment will result in pollution levels comparable to those associated with BPA.

However, BPF is not as safe as initially assumed, as several studies have demonstrated that BPF induces toxic effects similar to those induced by BPA. Both Kitamura et al. (2005) and Moreman et al. (2017) have shown that BPF has estrogenic activities that are similar to those of BPA, and Huang et al. (2016) reported that BPF induced thyroid endocrine disruption in zebrafish larvae. Moreover, the presence of BPF residues in foodstuffs raises concerns that BPF might also have anti-androgenic effects, as BPF is able to bind to androgen receptors (Cabaton et al., 2009; Roelofs et al., 2015). BPF has also been demonstrated to have genotoxic effects on chicken DT40 cells, although at lower rates than that of BPA (Lee et al., 2013). However, as Rosenmai et al. (2014) reported, BPF exhibits greater steroidogenic activity than BPA in human adrenal cortico-carcinoma cells *in vitro*. Nevertheless, although numerous studies have assessed BPF toxicity *in vitro* (Tišler et al., 2016), few studies have assessed its *in vivo* effects, particularly with regards to long-term exposure toxicities. Given the similarities in estrogenic activity and comparable pollution levels between BPF and BPA, further evaluations of the long-term environmental health impacts of BPF are needed.

The immune system is essential for managing environmental stressors (Graham Andrea et al., 2010), but numerous previous studies have shown that BPF can have detrimental effects on the immune system. For example, Dong et al. (2018) found that parental exposure to BPF influenced the immunity of zebrafish offspring, significantly inducing the activity of lysozyme and inhibiting genes associated with oxidative defense and respiratory burst response. Our previous *in vivo* and *in vitro* research clearly demonstrated that exposure to bisphenols at environmentally relevant concentrations has negative effects on immunomodulation in fish (Wu et al., 2011; Xu et al., 2013; Yang et al., 2015a; Qiu et al., 2016a, 2016c, 2018a, 2018b). A growing body of evidence suggests that BPF has the potential to induce oxidative stress and may have pro-inflammatory effects, and thus the potential immune disturbance of BPF has been implicated in a broad range of adverse effects. However, substantiation of the immune modulatory effects of BPF and its influence on the signaling pathways through which it exerts its mode of action (MOA) are limited. As such, our objective here was to determine whether BPF also modulated immunological activity.

The liver is responsible for immunological effects and the mononuclear phagocyte system of the liver contains many immunologically active cells that function as a “sieve” for antigens transported to it via the portal system. Because most immunoglobulins, acute phase proteins, complement components, and cytokines are synthesized in the liver (Nemeth et al., 2009), the liver of teleost fish species might therefore be an essential immune organ for processing immunotoxic environmental pollutants. We therefore used common carp (*Cyprinus carpio*) liver as an effective *in vivo* immune model to better evaluate the effects of BPF exposure on oxidation-reduction processes and immune system response. Several common immune parameters, including nitric oxide (NO) content and nitric oxide synthase (NOS) activity, complement component3 (C3) content, and immunoglobulin M (IgM) level, as well as oxidative stress indices, were measured following exposure to BPF at environmental levels over a period of 60 d. In addition, the nuclear factor-kB (NF-kB) signaling pathway is considered to be a key player in innate and adaptive immune responses, and functions as a central mediator of the inflammatory process (DiDonato Joseph et al., 2012), moreover, it has also been implicated in the action of bisphenols (Yang et al., 2015b). Thus, pro-inflammatory factors, including interleukin-1β, interleukin-6, interleukin-10, interleukin-11, interleukin-12 p35, cxc-chemokine, and cxcl-8, which were presumed to be genes associated with the NF-kB pathway (De Bosscher et al., 2006; Fujiki et al., 2003), were also evaluated in order to examine the involvement of NF-kB transcription factors in immunological responses to chronic BPF exposure.

2. Materials and methods

2.1. Chemicals

Both BPF (CAS Number 620-92-8, purity > 98%) and BPA (CAS number 80-05-7, purity > 99%) were obtained from Sigma-Aldrich (St. Louis, MO, USA). BPF and BPA were dissolved in dimethyl sulfoxide (DMSO) to obtain 10 g/L stock solutions, which were then stored at 4 °C for a period of 1 week. No more than 0.005% DMSO was present in the stock solutions.

2.2. Experimental design

Juvenile common carp (*Cyprinus carpio*), weighing on average 10–15 g, were allowed to acclimatize to laboratory conditions for 2 weeks and then randomly distributed into glass tanks (5 L) at a rate of five individuals per tank. On the basis of environmentally and ecologically relevant concentration of BPA (vom Saal and Welshons, 2006), the BPF exposure concentrations were 0.1, 1, 10, 100, and 1000 μg/L. A vehicle control group with 0.005% DMSO and a blank control group were set up in parallel. The exposure solutions were completely replaced daily and at least four replicates were used for each treatment group. Liquid chromatography-tandem mass spectrometry analysis indicated that less than 20% of the BPF was degraded under the experimental conditions.

We also compared the effects of BPF and BPA on immunotoxicity in the carp. Individual fish were exposed to 100 μg/L BPF and 100 μg/L BPA for 60 d, after which the survival rate, body weight, and body length of the fish were recorded. The concentration of 100 μg/L was selected based on the outcome concentration determined in two previous studies (Qiu et al., 2015, 2018a). Livers were removed from each fish and then weighed, and liver samples were snap-frozen in liquid nitrogen and stored at −80 °C. The hepatosomatic index (HSI) was also calculated using the formula:

$$HSI = \frac{liver\ \ weight}{body\ \ weight} \times 100\%.$$

2.3. Biochemical assays

Liver samples were homogenized in PBS (pH = 7.0), following which ROS content, total antioxidant capacity (T-AOC), catalase (CAT) activity, superoxide dismutase (SOD) activity, lipid peroxidation level (determined from the level of malondialdehyde), total NO level, induced NO synthase (iNOS) activity, complement component3 content, and IgM level were measured. All biochemical assays were performed with commercially available kits (Nanjing Jiancheng Bioengineering Institute).