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Bisphenol A and its analogs induce morphological and biochemical alterations in human peripheral blood mononuclear cells (*in vitro* study)

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ABSTRACT

Few studies have addressed the cellular effects of bisphenol S (BPS) and bisphenol AF (BPAF) on cells, and no study has been conducted to analyze the mechanism of action of bisphenols in blood cells. In this study, the effect of BPA, bisphenol F (BPF), BPS and BPAF on human peripheral blood mononuclear cells (PBMCs) was analyzed. It was shown that BPA, BPF and BPAF in particular, decreased cell viability, which was associated with depletion of intracellular ATP level and alterations in PBMCs size and granulation. Bisphenols enhanced ROS (including $OH^{\cdot}$) formation, which led to damage to lipids and proteins in PBMCs. The most significant alterations in ROS level were induced by BPF, and particularly BPAF. Moreover, it was shown that BPAF most strongly provoked lipid peroxidation, while BPA and BPS caused the greatest damage to proteins. It may be concluded that BPA and its analogs were capable of inducing oxidative stress and damage in PBMCs in the concentrations ranging from 0.06 to 0.5 μM (0.02–0.1 μg/ml), which may be present in human blood even as a result of environmental exposure. Although, most of bisphenols studied decreased cell viability, size and ATP level at higher concentrations, BPAF exhibited its cytotoxic potential at low concentrations ranging from 0.3 to 3 μM (0.1–1.0 μg/ml) that may correspond to concentrations in humans following occupational exposure.

1. Introduction

Bisphenols are aromatic compounds commonly used in various branches of industry. Those substances are mainly used in the synthesis of polycarbonate polymers and epoxy resins that are utilized in the production of varnishes, plastic containers, bottles, medical materials, lens, toys and other products (Michałowicz, 2014). Moreover, bisphenols are used in the production of thermal paper, which is employed in massive amounts in the production of register receipts, books, faxes and labels and also used (after recycling) to produce brochures, tickets, newspapers, kitchen rolls, toilet paper and food cartons (Liao et al., 2012a).

Bisphenol F (BPF) and bisphenol A (BPA) in particular are among the most commonly synthetized bisphenols. It was estimated that worldwide production of BPA was over 3 million tons in the 2003 (Vandenbergh et al., 2007). In the last decade, the production of other bisphenols has also significantly increased. For example, the production of bisphenol AF (BPAF) only in the USA has reached 250 tons (NTP, 2008). Similarly, worldwide production of bisphenol S (BPS) has raised significantly, which is associated with replacement of BPA with BPS in the production of numerous products made from polymers (including baby bottles and food containers) and thermal paper (Liao et al., 2012a; Lotti et al., 2013).

The exposure of the general population to bisphenols is mainly related with food consumption, particularly canned food in which BPA, BPF and BPS are contained in significant concentrations (11.5–317 μg/dm^3) (Yonekubo et al., 2008; Viñas et al., 2010). The exposure to bisphenols is also related with inhalation of these substances with dust present in indoor environments. Liao et al. (2012b) determined bisphenols (mainly BPA, BPF and BPS) in the concentrations ranging from 0.026 to 111 μg/g in dust present in houses, office and laboratory microenvironments. Moreover, it is considered that dermal contact with thermal paper containing BPA and BPS contributes to exposure of humans to bisphenols. Liao et al. (2012a) detected high concentrations of BPA and BPS in various paper products with the highest concentrations of 181 μg/g found in thermal paper and food cartons.

Abbreviations: BPA, bisphenol A; BPF, bisphenol F; BPS, bisphenol S; BPAF, bisphenol AF; PBMCs, peripheral blood mononuclear cells; FSC, forward scatter characteristics; SSC, side scatter characteristics; 6-carboxy-H_2DCFDA, 6-carboxy-2′,7′-dichlorodihydrofluorescein diacetate; HPF, 3′-(p-hydroxyphenyl)-fluorescein; PnAC, *cis*-parinaric acid; PCP, pentachlorophenol; 2,4,5-TCP, 2,4,5-trichlorophenol.

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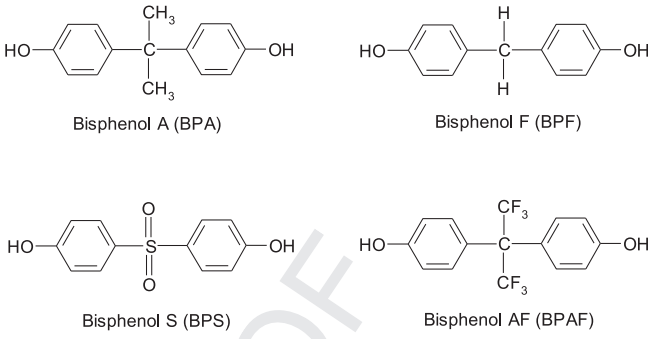
The common exposure of the general population to bisphenols leads to the occurrence of these compounds in humans. For instance, BPA was determined in blood serum of the USA population in the concentrations ranging from 0.4 to 149 ng/ml (Calafat et al., 2008). BPA was also determined in relatively high concentrations in humans occupationally exposed. In occupational setting high mean BPA concentration (approximately 5 μg/ml; 5.4 μg/g creatinine) were detected in the urine of Chinese workers employed in the production of epoxy resins (He et al., 2009). The results obtained by Liao et al. (2012c) showed the common exposure of the populations of the USA, China, India and other Asian countries to BPS. They determined BPS in 81% of urine samples in the concentrations ranging from trace to 21 ng/mL with the highest amounts detected in the citizens of highly urbanized countries including USA and Japan.

Although, the analysis of the presence of BPAF in human body has not been conducted up to now, the results of the investigations have shown significant bioaccumulation potential of this substance. It was noticed that BPAF given orally to rats was accumulated in various tissues and serum of the animals studied with the highest concentrations determined in liver, kidneys and adipose tissue (Yang et al., 2012).

The obtained results have shown that BPA reveals estrogenic activity in humans (Li et al., 2010; Melzer et al., 2011). It also promotes leukemia and lymphoma development in rats and possibly causes prostate gland and nipple cancers development in humans (Benachour and Aris, 2009; Li et al., 2010). The investigations have also revealed that BPF causes breast cancer development and negatively influences reproductiveness in rats (Coleman et al., 2003; Cabaton et al., 2006). Other studies have shown that BPA exhibits immunotoxic potential. Richter et al. (2007) observed that BPA modulated production of cytokines by lymphocytes T of rodents, which led to improper response of the immune system. The investigations conducted on mouse splenocytes showed that BPA modulated lymphocytes B proliferation and production of some cytokines and antibodies (Wetherill et al., 2007). In epidemiological study realized by National Health and Nutrition Examination Survey (USA), it was suggested that BPA negatively influenced immune function in children and adults (Clayton et al., 2011).

The results of toxicological studies that focused on toxic effect of BPAF and BPS are much more limited. In some countries, BPA was replaced with BPS in the production of baby bottles and food containers, which was due to concern about the toxicity of BPA. Nevertheless, lack of basic researches concerning toxic effect of BPS on humans makes impossible to evaluate rightness of this decision. Answer for this question is more and more important in the light of the results obtained by Grignard et al. (2011) who observed that BPS exhibited similar estrogenic activity to that exerted by BPA. BPAF has also been proven to reveal significant toxicity. For example, Tsutsui et al. (2000) proved that BPAF exhibited stronger toxicity than BPA in Chinese hamster ovary cells and Kitamura et al. (2005) showed that BPAF caused stronger inhibitory effect than BPA on the androgenic activity of 5α-dihydrotestosterone in mouse fibroblast cell line. Necessity of continuation of toxicological studies concerning BPAF has also been noticed by other scientists. In 2008, the U.S. National Institute of Environmental Sciences nominated BPAF for comprehensive toxicological characterization based on the lack of toxicity data. This decision was also undertaken due to results of initial investigations, which evidenced non-occupational and occupational exposure of people to BPAF and predicted toxicity of this substance (NTP, 2008).

In the light of the above data, we decided to compare the effect of BPA, BPF, BPS and BPAF on necrotic and morphological changes in human peripheral blood mononuclear cells (PBMCs), which are suitable model to analyze xenobiotics toxicity. Moreover, we evaluated changes in ATP level, reactive oxygen species and hydroxyl radical formation as well as protein and lipids damage in PBMCs exposed to BPA and its analogs.



Chemical structures of bisphenols

2. Materials and methods

2.1. Chemicals

Bisphenol A (99%, 2,2-bis(4-hydroxyphenyl)propane) (BPA) (CAS No. 80-05-7), bisphenol F (99%, 4,4′-dihydroxydiphenylmethane) (BPF) (CAS No. 620-92-8), bisphenol S (99%, 4,4′-sulfonyldiphenol) (BPS) (CAS No. 80-09-1), bisphenol AF (99%, 2,2,-bis(4-hydroxyphenyl)hexafluoropropane) (BPAF) (CAS No. 1478-61-1), calcein-AM (95%, CAS No. 148504-34-1), propidium iodide (95%, CAS No. 25535-16-4), 6-carboxy-2′,7′-dichlorodihydro-fluorescein diacetate (H_2DCFDA) (95%, CAS No. 4091-99-0), ethanol (ACS grade; CAS No. 64-17-5) and fetal bovine serum (FBS) were bought from Sigma–Aldrich (USA). Bioluminescence assay kit for ATP determination, 3′-(p-hydroxyphenyl)-fluorescein (HPF) (98%, EC No. 200-679-5) and *cis*-parinaric acid (CAS No. 593-38-4) were bought from Molecular Probes (USA). Lymphocyte separation medium (LSM) (1.077 g/cm^3) and RPMI with L-glutamine were bought from Cytogen (Germany). Sodium chloride (99.5%, CAS No. 7647-14-5), potassium chloride (99.5%, CAS No. 7447-40-7), ammonium chloride (99.5%, CAS No. 12125-02-0), sodium hydrogen carbonate (99%, CAS No. 97328-76-2), sodium wersenite Na_2EDTA (99.5%, CAS No. 60-00-4) and other chemicals were purchased from Roth (Germany) and POCH (Poland).

2.2. PBMCs isolation and treatment

Leucocyte buffy-coat was collected by Blood Bank in Łódź, Poland. Blood was obtained from 20 healthy, non-smoking volunteers (aged 18–55), who showed no signs of infection disease symptoms at the time the blood samples were collected. PBMCs were isolated using LSM (1.077 g/cm^3) by centrifugation at 600*g* for 30 min at 20 °C. PBMCs were collected, suspended in erythrocyte lysis buffer (150 mM NH_4Cl, 10 mM $NaHCO_3$, 1 mM EDTA, pH 7.4) and incubated for 5 min at 20 °C. Then, PBS was added immediately, and the cells were centrifuged at 200*g* for 15 min at 20 °C. The supernatant was decanted, and the cells were washed twice with RPMI with L-glutamine and 10% fetal bovine serum (FBS) at 200*g* for 15 min. The cells were resuspended in RPMI medium with L-glutamine and 10% FBS and counted in haemocytometer. The final PBMCs density used in the experiments (after addition of bisphenol solution) was 1×10^6 cells/ml. The viability of the cells was over 95%.

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