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ORIGINAL ARTICLE

Bisphenol A as an environmental pollutant with dual genotoxic and DNA-protective effects

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Abstract

OBJECTIVES: Bisphenol A (BPA) is an endocrine disruptor which has been shown to be a harmful compound for living organisms. It is the main component of the most commonly used plastic products such as plastic bottles, food cans and containers or dental fillings, and other medical aids. Recently, it has become a new environmental pollutant. The current knowledge about the BPA effects (including genotoxic one) on different cells is in many cases contradictory. Thus, the aim of the paper is to study the potential genotoxic effect of BPA.
METHODS: An observation of the genotoxic activity of BPA on human lymphocytes was evaluated by using the alkaline comet assay and a modified comet assay with bacterial DNA repair enzyme Fpg. The potential DNA-protective effect of BPA was tested by using the DNA-topology assay.
RESULTS: The results show that rising concentrations of BPA increase the risk of DNA double-strand breaks and modified purines in human lymphocytes. Interestingly, BPA shows an ability to protect plasmid DNA from the damage of iron ions in cell-free system.
CONCLUSIONS: BPA itself does not induce genotoxic effect to DNA. However, BPA treatment of human lymphocytes leads to the induction of DNA damage. The proposed mechanism of BPA action in the human lymphocytes could be mediated by cell metabolism that induces an oxidative stress and ROS formation. ROS subsequently attack DNA and thus induce DNA damage. According to our results, BPA can be included in the group of substances with dual effects involving genotoxic and DNA-protective activity.

INTRODUCTION

Bisphenol A (BPA, 2,2-bis(4-hydroxyphenyl) propane) is a chemical compound, widely used in industry to manufacture polycarbonate plastic, epoxy resins and thermosensitive paper (Kubwabo *et al.* 2009). It is synthesized by acid catalyzed condensation of acetone and phenol (Prokop *et al.* 2004). Polycarbonate plastics serve to produce many products of everyday use, such as plastic bottles, food and drink cans, food containers, CDs, DVDs or some toys (Vandenberg *et al.* 2007). Nowadays it is forbidden to use BPA in the production of baby bottles. BPA derivatives are often used in medical aids, especially in dental fillings (Fleisch *et al.* 2010). BPA was first devel-

oped in 1891 by Russian chemist Aleksandr P. Dianin as an organic synthetic estrogen, which was reported to have the efficacy of estrone, a minor female sex hormone. Its primary use in the 1930s was to stimulate the female reproductive system in rats, and later BPA was more known for its use in the manufacturing of polycarbonate plastic and epoxy resins (Dodds & Lawson 1936). At present, BPA is often used in industry for its ability to give plastic products desired characteristics (strength, hardness, toughness, transparency, resistance to temperatures between about –40 °C and about 145 °C, and resistance to many acids and oils) (Staples *et al.* 1998).

BPA belongs to endocrine disrupting chemicals (EDCs) which are exogenous substances that can alter the function of the endocrine system and as a result of this, it can cause adverse effects in intact organisms. EDCs interact with estrogen receptors and act as an agonist or an antagonist via estrogen receptor-dependent signalling pathways (Matthews *et al.* 2001). Thus, by its ability to bind to estrogen receptors, BPA can mimic biological signals and affects the functions of some endogenous hormones, thereby contributing to hormonal imbalance in the body. Undesirable physiological effects and disruption of natural pathways going on in the body may be the consequences of the BPA treatment (Takayanagi *et al.* 2006; Hwang *et al.* 2011; Rubin 2011; Ribeiro-Varandas *et al.* 2012; Ferguson *et al.* 2016). BPA was confirmed to cause the malignant transformation of healthy cells into breast, prostate or brain cancer cells, responsive to changes in behavior or reproduction (Mikołajewska *et al.* 2015; Paulose *et al.* 2015; Ferguson *et al.* 2016; Santangeli *et al.* 2017). BPA also contributes to the development of diabetes, neurodegenerative diseases (Alzheimer`s disease, Parkinson`s disease), cardiovascular diseases and abnormal levels of liver enzymes (Kubwabo *et al.* 2009; Rubin 2011).

Besides being the endocrine disruptor, BPA can cause oxidative stress in the body (Ferguson *et al.* 2016). Oxidative stress is defined as an imbalance between the production of reactive oxygen species (ROS, free radicals) and the number of antioxidants in the body (Betteridge 2000). Residual BPA enzymatically or non-enzymatically could induce the formation of phenoxy radicals which subsequently react with NADPH or intracellular glutathione (GSH), resulting in ROS formation (Sakuma *et al.* 2010; Babu *et al.* 2013).

An excessive increase of using BPA in everyday life can potentially endanger human health and also the environment. Thus, there is good reason to evaluate effects of this compound on living organisms, especially on humans. Unfortunately, the potential genotoxicity and DNA-damaging activity and/or DNA-protectivity of BPA is still not clear. The present study was designed to investigate the potential genotoxic effect of BPA using the alkaline comet assay and the modified comet assay with Fpg enzyme. On the other hand, the potential DNA-protective and/or DNA-damaging effects of BPA were evaluated by using method enabling to monitor the changes in the plasmid DNA topology.

MATERIAL AND METHODS

Isolation of human lymphocytes

Human lymphocytes were collected from human peripheral blood obtained by *finger prick* method followed by centrifugation in a density gradient of Histopaque *(*SigmaAldrich*)* medium (3 min, 2 g, 4°C).

Cell treatment

Lymphocytes obtained from the Histopaque layer mentioned above were mixed with 1 ml of PBS (phosphate buffer solution), centrifuged under the same condition, immobilized in low melting point agarose on pre-coated glass slides and treated with BPA *(*SigmaAldrich*)* dissolved in H_2O (with adjusted pH to 7.0) for 1 h at 37 °C. As a positive control, 400 μM H_2O_2 for 5 min at 4 °C was used. As negative controls, the cells were either left untreated in fresh PBS buffer solution for 1 h at 37 °C, or were treated with H_2O pH 7.0 for 1 h at 37 °C.

Alkaline comet assay and modified comet assay with Fpg enzyme

Single-cell gel electrophoresis was performed under alkaline conditions according to the procedure of Singh *et al.* (1988) with modifications of Collins *et al.* (1993) without or with the inclusion of a digestion with bacterial enzyme Fpg (formamidopyrimidine-DNA glycosylase, BioLabs, New England) after the lysis of cells in the gel (Collins *et al.* 1997).

DNA-topology assay

The electrophoretic method for potential DNA-damaging and DNA-protective activity monitoring was previously described in Sevcovicova *et al.* (2015). The reaction contained 300 ng of plasmid DNA (pBR322, BioLabs, New England) and either Fe^{2+} ions (1 mM $FeSO_4{\cdot}7H_2O$, Lachema) alone, or BPA alone, or combinations of BPA with Fe^{2+} ions. Topological changes of pDNA molecules correspond with the electrophoretic mobility of pDNA topoisomers. DNA breaks were assayed by measuring the conversion of supercoiled pDNA to relaxed circular DNA or to linear DNA. An analysis of pDNA lesions was made by the agarose gel electrophoresis. The pDNA fragments were visualized by staining with StopC dye (xylene cyanol, bromphenol blue, both 1 mg/mL) and UV illumination system (UV Transilluminator MiniBISPro, DNR Bio Imaging Systems Ltd.).

Statistical analysis

The comet assay results represent the mean of three experiments ± standard deviation. The significance of differences between the means was evaluated by the Student's t-test: ** (##) $p<0.01$; *** (###) $p<0.001$.

RESULTS

The DNA strand breaks level and the level of oxidative DNA lesions induced by BPA

The potential genotoxic effect of BPA in a concentration range 0.001–2.5 mM was investigated by using the alkaline comet assay on human lymphocytes (Figure 1). The alkaline comet assay is a sensitive method for the potential assessment of primary DNA damages. It can be used to detect single- and double-strand breaks, alkali-labile sites that are caused as single-strand breaks, and single-strand breaks associated with an incomplete excision repair. Under certain conditions, the assay can also detect DNA-DNA and DNA-protein crosslinking, which (in the absence of other kinds of DNA lesions) appears as a relative decrease in DNA migration compared with concurrent controls (Hartmann *et al.* 2003). As illustrated Figure 1 (black columns), BPA without Fpg treatment increased the percentage of damaged DNA compared to negative controls in the dose-response manner. The highest concentration of BPA (2.5 mM) induced the similar amount of DNA damages as 0.1 mM BPA probably due to overlaping of the mutagenic effect with toxicity. Our results correspond with other scientific teams` results. Xin *et al.* (2014) evaluated the level of DNA strand-breaks in INS-1 cells exposed to BPA (0–100 μM) by using the comet assay. They found out that BPA causes DNA damage in a dose-dependent manner. Xin *et al.* (2015) found out that BPA could exhibit significant dose-dependent genotoxic effect in Chinese hamster ovary cells in all tested concentrations (40–120 μM).

Oxidative DNA lesions induced by prooxidant effect can be recognized by a lesion-specific enzyme, for example Fpg, which catalyses the excision of damaged purines in DNA (Gabelova *et al.* 1997; Krokan *et al.* 1997). A repair of oxidized bases is difficult to study, since standard biochemical methods are not sufficiently sensitive to measure individual oxidation products formed after biologically tolerable doses of damage. Collins *et al.* (1993) modified the comet assay with the use of purified DNA repair enzymes in order to detect specific types of DNA damage. Using modified comet assay with Fpg enzyme we observed an increase of DNA double-strand breaks in BPA-treated human lymphocytes at all concentrations used. The effect was most noticeable at the lowest concentration (0.001 mM BPA) compared to negative controls (Figure 1, white columns). The increase of DNA double-strand breaks has demonstrated that BPA induces oxidation damage to DNA of human lymphocytes.

DNA-topology assay

The DNA-topology assay responds sensitively to an occurrence of a metal and related free-radical generation in the reaction medium (Cipak *et al.* 2001). The results presented in Figure 2 indicate that BPA alone (Figure 2a) did not change the DNA mobility in the

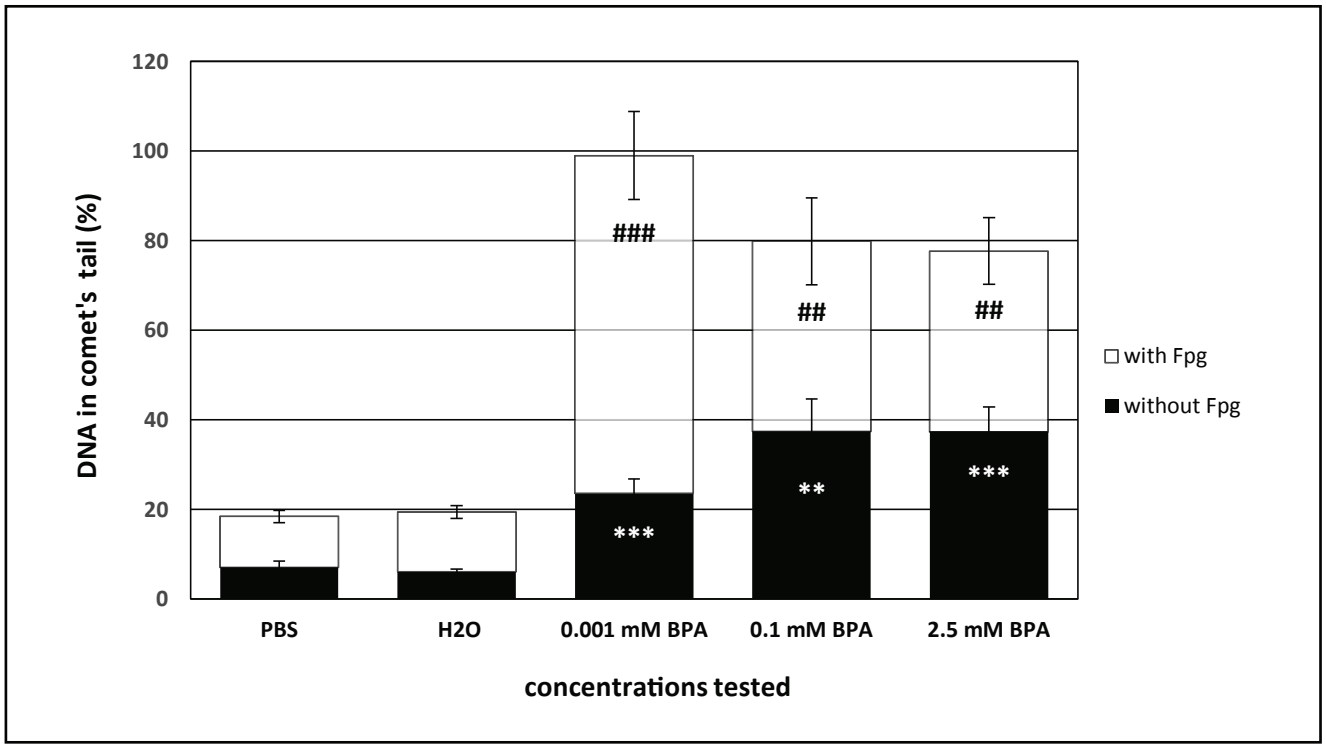

Fig. 1. The level of DNA-strand breaks and Fpg-sensitive lesions in human lymphocytes after BPA treatment. Black columns represent the rising amount of DNA breaks caused by increasing BPA concentration without Fpg treatment. On the other hand, white columns represent the rising amount of double-strand DNA breaks after Fpg treatment. Negative controls: PBS (phosphate buffer solution) and H_2O (with adjusted pH for BPA to dissolve).

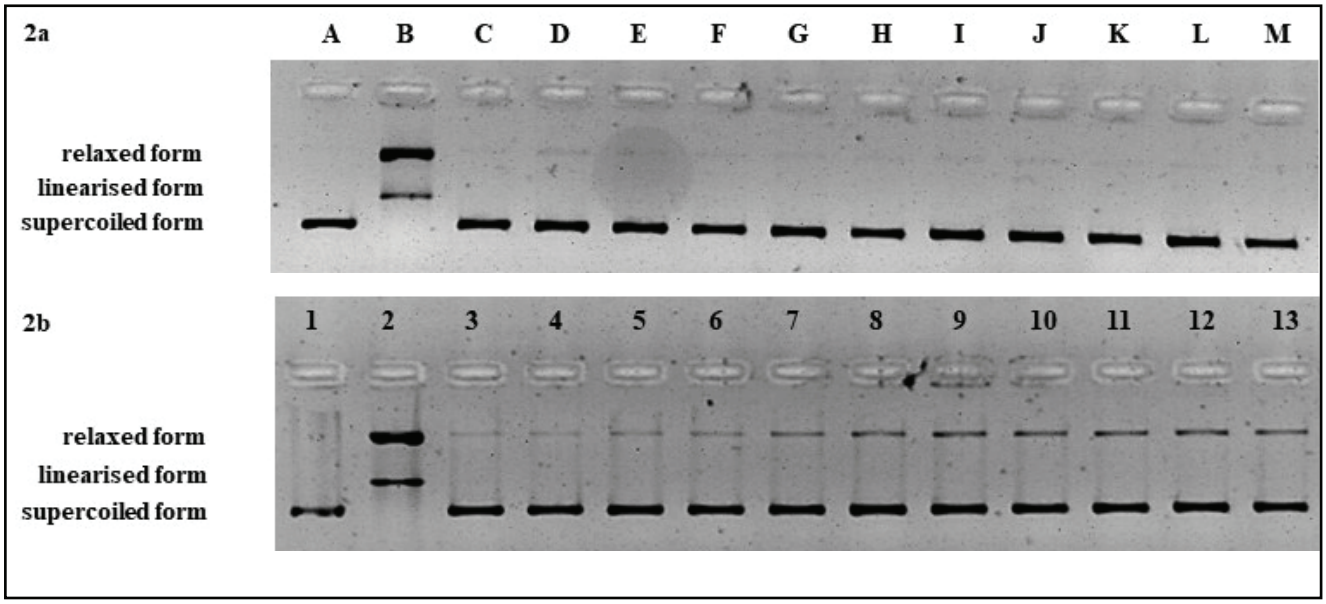

Fig. 2. DNA topology assay. BPA itself does not cause damage of pDNA (2a). Interestingly, with increasing concentrations, BPA has protected pDNA against Fe^{2+} ions induced oxidative damage (2b). Lanes A, 1: negative control (native pBR322); lanes B, 2: positive control (pBR322 + Fe^{2+} ions); lanes C–M: decreasing concentrations of BPA: 10 mM; 5 mM; 2.5 mM; 1 mM; 0.1 mM; 0.01 mM; 0.001 mM; 10^{-4} mM; 10^{-5} mM; 10^{-6} mM; 10^{-7} mM; all samples without $FeSO_4 \cdot 7H_2O$; lanes 3–13: the same concentrations of BPA; all samples with $FeSO_4 \cdot 7H_2O$.

agarose gel. Surprisingly, we proved BPA DNA-protective activity in the presence of Fe^{2+} ions in the reaction medium in a dose-dependent manner (Figure 2b).

DISCUSSION

Based on the comet assay and the modified comet assay results, we can conclude that BPA induces DNA-breaks in human lymphocytes, mostly as a consequence of oxidative damage to DNA. Our results are in accordance with the results of another study in which the increased amount of oxidative DNA damage was confirmed in salesmen, who are in daily contact with BPA-containing thermosensitive paper (Lv *et al.* 2017). Thus, the possible mechanism of BPA action by indirect way could be based on the induction of oxidative stress in cells after the BPA treatment. BPA itself cannot damage plasmid DNA in cell-free systems and moreover, in higher concentrations could even protect plasmid DNA against iron ions induced single-strand DNA breaks as showed by DNA-topology assay. Contrary to this, BPA manifests a strong genotoxic effect on human lymphocytes in the comet assay. We suppose that the genotoxic effect of BPA is mediated by the cell metabolism, which might induce ROS after the treatment of lymphocytes with BPA. Therefore, BPA can be included in the group of substances with dual effects involving genotoxic and DNA-protective activities. Our findings confirm that the study of BPA and genotoxic safety of other environmental pollutants is still up-to-date and needed in order to prevent the harmful effects of these pollutants on population health.

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