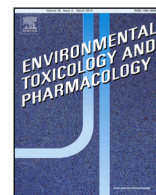
Environmental Toxicology and Pharmacology 40 (2015) 524–529

Contents lists available at ScienceDirect

Environmental Toxicology and Pharmacology

journal homepage: www.elsevier.com/locate/etap



# Cytogenetic evaluation for the genotoxicity of bisphenol-A in Chinese hamster ovary cells

Lili Xin[a,b,1], Yao Lin[c,1], Aiqing Wang[c], Wei Zhu[d], Yu Liang[e], Xiaojun Su[e], Chengjiao Hong[c], Jianmei Wan[c], Yarong Wang[c], Hailin Tian[a,b,*]

[a] School of Public Health, Medical College of Soochow University, 199 Renai Road, Suzhou 215123, Jiangsu, China
[b] Jiangsu Key Laboratory of Preventive and Translational Medicine for Geriatric Diseases, School of Public Health, Soochow University, Suzhou 215123, China
[c] Experimental Center of Medical College, Soochow University, 199 Renai Road, Suzhou 215123, Jiangsu, China
[d] School of Radiation Medicine and Protection, Medical College of Soochow University, 199 Renai Road, Suzhou 215123, Jiangsu, China
[e] Sanitation & Environment Technology Institute, Soochow University, 199 Renai Road, Suzhou 215123, Jiangsu, China

ARTICLE INFO

*Article history:*
Received 15 April 2015
Received in revised form 4 August 2015
Accepted 5 August 2015
Available online 7 August 2015

*Keywords:*
Bisphenol A
Endcorine disruptor
DNA damage
Micronucleus
Cytogenetic
Mutagenicity

ABSTRACT

Bisphenol A (BPA), identified as an endocrine disruptor, is an important man-made compound used in a wide range of consumer products. The MTT assay, comet assay, micronucleus test, chromosome aberration test, and Ames assay were conducted to assess the cytotoxic, genotoxic, cytogenetic effects, and mutagenic activity of BPA. After BPA exposure, we showed significant increases in cytotoxicity and level of DNA damage indicated by Olive tail moment, tail length, and % tail DNA in a similar dose- and time-dependent manner. Significant increases in micronucleus frequency and conventional chromosome aberrations were also observed after BPA treatment. The major types of structural aberrations were breaks, gaps, and fragments. However, no positive mutagenic activity of BPA was observed in any of the tester strains. Taken together, the data obtained in this study clearly demonstrated that BPA is not mutagenic but could exhibit significant genotoxic and cytogenetic effects in Chinese hamster ovary cells.
© 2015 Elsevier B.V. All rights reserved.

## 1. Introduction

Increasing concentrations of endocrine disruptors (EDs) are emerged as major public health problems because of their capacities to behave as biological signals and interfere with estrogenic hormones (Ribeiro-Varandas et al., 2013). Among the various EDs, bisphenol-A (BPA) is the most important man-made compound with annual production exceeding 3.8 million tons (Michalowicz, 2014). BPA is mainly employed in the manufacture of a variety of consumer products such as polycarbonate plastic and resins, medical tubing, toys, water pipes, and dental sealants (Huang et al., 2012). Besides, it is also can be used as liners for food packaging, bottles, and coatings for tins, thus leading to the exposure of consumers to BPA through food and drinking water (Makris et al., 2014). Due to its ubiquitous and widespread distribution, BPA has provoked worldwide concerns about its possible associations with human diseases such as diabetes, obesity, cardiovascular disease, and cancer (Jenkins et al., 2011; Yang et al., 2006).

As an Endocrine Disrupting Chemical, BPA is able to bind to several kinds of receptors including estrogen and androgen. Because the chemical structure of BPA is similar to that of carcinogenic diethylstilbestrol, an important issue on the genotoxic and carcinogenic activities of BPA has come forward. Although the possible genotoxicity of BPA has been widely tested in various *in vitro* or *in vivo* studies, most of the results are controversial. Several studies have shown that BPA can induce chromosome aberrations and DNA adducts formation in Syrian hamster embryo (SHE) cells (Tsutsui et al., 1998) or micronuclei formation in human MCL-5 cells (Parry et al., 2002). Aneuplodoidogenic properties were also observed in SHE cells and Chinese hamster V79 cells after BPA exposure (Pfeiffer et al., 1997; Tsutsui et al., 1998). On the other hand, BPA failed to induce gene mutations in both SHE and V79 cells (Schweikl et al., 1998; Tsutsui et al., 1998). Pacchierotti et al. (2008) showed that BPA did not result in any significant induction of hyperploidy, polyploidy or micronucleus in male mice. These discrepancies warrant the need for further studies on the genetic toxicity of BPA, and such

*Abbreviations:* EDs, endocrine disruptors; BPA, bisphenol-A; SHE, Syrian hamster embryo; SCGE, single cell gel electrophoresis; CHO, Chinese hamster ovary; LMPA, low melting point agarose; NMPA, normal melting point agarose; DMSO, dimethyl sulfoxide.

* Corresponding author at: School of Public Health, Medical College of Soochow University, 199 Renai Road, Suzhou 215123, China. Tel.: +86 512 65880070; fax: +86 512 65880070.
*E-mail address:* hltian@suda.edu.cn (H. Tian).
[1] Lili Xin and Yao Lin contributed equally to this work.

http://dx.doi.org/10.1016/j.etap.2015.08.002
1382-6689/© 2015 Elsevier B.V. All rights reserved.

studies will be valuable in human risk assessment of environmental estrogens.

The aim of this study was to assess the possible genotoxic effects of BPA exposure by measuring the DNA damage using single cell gel electrophoresis (SCGE or Comet) assay, micronucleus frequency, and structural chromosome aberrations in Chinese hamster ovary (CHO) cells. The mutagenicity of BPA was also evaluated using tester strains of *Salmonella typhimurium* (TA97, TA98, TA 100, TA102, and TA1535) in the presence and absence of metabolically active microsomal fractions (S9).

## 2. Materials and methods

### *2.1. Chemicals and materials*

Bisphenol-A (BPA) was purchased from Tianjin Guangfu Fine Chemical Research Institute (Tianjin, China). The RPMI-1640 medium and newborn calf serum were obtained from GIBCO (Grand Island, NY, USA). The normal and low melting point agarose (NMPA and LMPA) was purchased from AMRESCO (Solon, OH, USA). Dimethyl sulfoxide (DMSO) was obtained from Sinopharm Chemical Reagent Co. Ltd in China. Trypsin, ethidium bromide (EB), 9-aminoacridine (9-AA), 2-nitrofluorine (2-NF), 2-aminoanthracene (2-AA), and 2-aminofluorene (2-AF) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Colchicine was obtained from Shanghai Keda Biomedical Science and Technology Company (Shanghai, China). Sodium azide ($NaN_3$) was purchased from Alfa Aesar (London UK). Mitomycin C (MMC) was obtained from Roche Group (Basel, Switzerland).

### *2.2. Cell culture*

The CHO cells were kindly provided by Department of Cell Biology, Medical College of Soochow University. Cells were cultured in RPMI-1640 culture medium supplemented with 10% newborn calf serum. Cultures were maintained in a humidified atmosphere with 5% $CO_2$ at 37 °C and medium was refreshed every 1 or 2 days with sub-culturing.

### *2.3. Cell treatment*

Prior to each experiment, CHO cells were seeded in triplicate at a density of $0.5 \times 10^4$ per well in 96-well culture plates or $2.5 \times 10^5$ per well in 6-well culture plates. After 24 h, the culture medium was replaced with fresh medium supplemented with vehicle alone (DMSO), increasing concentrations of BPA (40, 80, 100, 120 μM) for 12 or 24 h (for MTT and comet assays) or 80, 100 and 120 μM BPA for 24 h (for micronucleus and chromosome aberration tests). The BPA was dissolved in DMSO and the concentration of the stock solutions was 300 mM. The final concentrations of DMSO used in all experiments did not exceed 0.1%.

### *2.4. Cell viability assay*

The number of viable CHO cells in culture was detected using the MTT assay. The cell viability was determined by measuring the capacity of the CHO cells to reduce the tetrazolium salt, MTT, to a blue formazan product (Denizot and Lang, 1986). The cells were seeded in 96-well plates under different treatments in DMEM culture medium with 10% newborn calf serum. Then, the cells were incubated with MTT (500 μg/mL) at 37 °C for 4 h, and lysed with DMSO. Finally, the absorbance was measured at 570 nm using the SYNERGY 2 microplate reader (Bio-Tek, USA). The untreated cells were the 100% viable control.

### *2.5. Comet assay*

The comet assay was carried out under the alkaline conditions using the method previously described by Singh et al. (1988) with some modifications. Briefly, the frosted slides were prepared and covered with 110 μL of 1.0% NMPA. After solidification, cell suspension and 0.8% LMPA were mixed well as the top layer of slides. After 20 min of solidification, the slides were immersed in lyses buffer for 1.5 h at 4 °C in dark. Then, the slides were washed and equilibrated in the alkaline buffer in order to unwind DNA. The electrophoresis was carried out for 30 min at 25 V and 300 mA, and then the slides were rinsed 3 times with neutralization buffer and stained with 5 mg/L EB. 100 random selected cells of each sample were analyzed according to Comet Assay Software Project (CASP, http://casplab.com/). The means of Olive tail moment, tail length, and % tail DNA were recorded to evaluate the levels of DNA damage.

### *2.6. Micronucleus test*

After treated with different concentrations of BPA for 24 h, the CHO cells were harvested and suspended with 10 mL of 75 mM KCl for 50 s at room temperature. The cells were fixed in 5 mL of Carnoy's solution (1:3 mixtures of acetic acid and methanol) for 30 min. Then, the cells were dropped onto clean microscopic slides, air-dried and stained with Giemsa. A total of 1000 cells for each sample were examined microscopically, and the micronuclei frequency was reported as the percentage of micronuclei cells per 1000 cells.

### *2.7. Chromosome aberration test*

After 4 h treatment of 20 μg/mL Colcemid, the CHO cells were harvested and suspended with 75 mM KCl for 30 min followed by centrifugation at 2000 rpm for 10 min. Then, the cells were fixed twice in Carnoy's solution, resuspended in fixative and dropped onto chilled slides. Finally, the slides were air-dried and stained with 10% Giemsa. Well spread 500 metaphases for each group were examined to score the aberrations according to the international system for Human Cytogenetic Nomenclature system (Harden and Klinger, 1985). Chromosomal aberrations including chromatid-type and chromosome-type breaks were enumerated after BPA exposure.

### *2.8. Ames assay*

A stock solution of BPA was prepared prior to the test by dissolving (1.24 g) of BPA in 1 mL of DMSO. Aliquots of different concentration such as 10, 30, 90, 180, 270, 810, 1620, 2430, and 5000 μg were tested in *S. typhimurium* tester strain TA97, TA98, TA100, TA102, and TA1535 using plate pre-incubation method in triplicates. The mixtures consisting of bacteria and test compound were plated in soft agar on histidine deficient agar plates. These plates were then incubated at 37 °C for 48 h and the revertant colonies were counted. The experiment was carried out in the presence and absence of a microsomal fraction (S9) from rat liver. Negative vehicle controls and positive controls in the absence/presence of S9 were included for ascertaining response of known compound in each assay. Positive controls were 9-AA (for TA97 without S9), 2-NF (for TA98 without S9), $NaN_3$ (for TA100 and TA1535 without S9), MMC (for TA102 without S9), 2-AF (for TA97, TA98, TA100 and TA102 with S9), and 2-AA (for TA1535 with S9); they were all prepared in DMSO. Three independent experiments were carried out and each dose was tested in triplicates.

Download English Version:

https://daneshyari.com/en/article/2582924

Download Persian Version:

https://daneshyari.com/article/2582924

Daneshyari.com