J. Fac. Pharm. Istanbul / İstanbul Ecz. Fak. Derg.
46(2) 2016 pp.97-114

Original Article

Alteration on global and gene-spesific DNA methylation and global histone modifications in HepG2 cells in response to BPA

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Abstract: Bisphenol A (BPA), as synthetic monomer used in industry in the production of polycarbonate plastic and epoxy resins, has endocrine disruptor properties and high risk on human health. Continuous release of free BPA into food, beverages, and the environment has resulted in a widespread human exposure to this chemical. Recent studies have showed the role of endocrine effects of environmental chemicals on the changes in gene expression may be associated with epigenetic mechanisms such as DNA methylation and histone modifications. The aim of the study was to investigate dose-related effects of BPA (0, 0.1 μM, 1 and 10 mM for 48 and 96 h) on global and gene-spesific (*p16, cyclin D2* and *Rassf1* genes) DNA methylation in human hepatocarcinoma (HepG2) cells. We also investigated global histone modifications such as H3K9 trimethylation (H3K9me3), H3K9 acetylation (H3K9ac), H3K27 trimethylation (H3K27me3). The 50% inhibitory concentration (IC_{50}) value of BPA was determined as 134 and 180 μM in HepG2 cells for 24 h by MTT and neutral red uptake (NRU) tests, respectively. We observed decrease on the global levels of 5-methylcytosine and 5-hydroxymethylcytosine at 1 and 10 mM after 96 h BPA exposure. There is no significant alterations on the promoter-region of methylation and expression of *p16, cyclin D2* and *Rassf1* genes. Global levels of H3K9me3 decreased after 0.1 and 1 μM concentration of BPA exposure for 48 h, while increased after 96 h 0.1 and 1 μM of BPA exposure for. Hovewer, it has not been observed significantly changes for the global levels of H3K27me3 and H3K9ac. In this study we suggest that BPA may disrupt epigenetic events by altering global and gene-spesific DNA methylation and histone modifications in HepG2 cells.

Key words: Bisphenol A, DNA methylation, cytotoxicity, histone modifications, HepG2 cells.

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Introduction

BPA has been used as synthetic monomer in production of polycarbonates and plastics in the food contact materials such as re-usable plastic tableware and epoxy resin in industry since 1950s. BPA is also used in a number of non-food-related applications such as epoxy resin-based paints, medical devices, surface coatings, printing inks, thermal paper and flame retardants and also in plastic materials such as CDs, DVDs (EFSA, 2015). Migration of BPA residues into food, beverages, and the environment has resulted in human exposure to this chemical (EFSA, 2015). BPA is a known endocrine disruptor. It has been suggested that BPA may influence multiple endocrine-related pathways (Rubin, 2011).

In recent years, it has been thought that BPA was related to ethiology of several chronic diseases such as diabetes, obesity, cardiovascular diseases, reproductive abnormalities, developmental disorders and breast cancer. It was shown that BPA exposure and mechanisms associated with its relationship with chronic diseases could be related to oxidative stress, mitochondrial dysfunctions, genetic, epigenetic and endocrine disruption mechanisms including cell signaling pathways (Rezg et al., 2014). Additionally, *in vitro* and *in vivo* studies have shown that BPA caused reproductive and developmental toxicity such as infertility, miscarriage and stillbirths (Takahashi & Oishi, 2001; George et al., 2008; Agarwal et al., 2016).

Epigenetics is defined as changes in gene expression without alterations of DNA sequences which encompass the mechanisms of DNA methylation, histone modifications, and non-coding miRNA regulation. Destroying these mechanisms has caused genomic instability. Effects of BPA on epigenetic mechanisms such as global and gene-specific DNA methylation, histone modifications, the expression level of chromatin-modifying genes in liver have been reported in several studies (Faulk et al., 2015; Weinhouse et al., 2015; Faulk et al., 2016; Ke et al., 2016; Laing et al., 2016). Especially abnormal hypermethylation of promoter regions in tumor suppressor genes is a key event in the formation and progression of cancer. *p16*, *cyclin D2* and *Rassf1* genes are tumor suppressor genes and involved in cell cycle regulation. These genes are significantly important for several cancer types such as lung and breast cancer, urothelial and hepatocellular carcinoma (Belinsky et al., 1998; Burbee et al., 2001; Toyooka et al., 2001; Zöchbauer-Müller et al., 2001; Pu et al., 2006; Jueliger et al., 2016).

Therefore, we aimed to investigate the effects of BPA on gene expressions and histone modifications to determine if aberrant DNA methylation and changes of gene expression levels and histon modifications are an early feature of endocrine disrupting chemical toxicity in the human hepatocellular carcinoma (HepG2) cell line.

Materials and methods

Chemicals

BPA (99% purity) was obtained from Sigma-Aldrich (St Louis, Missouri, USA). A stock solution of BPA (1000 µM) was prepared dissolving it in dimethyl sulfoxide (DMSO, Sigma-Aldrich, St Louis, Missouri, USA) in a sterile glass volumetric flask and kept at -20°C. Cell culture media and all other supplements were purchased from Wisent Bioproducts (Saint-JeanBaptiste, QC, Canada) and sterile plastic materials were purchased from Greiner (Frickenhausen, Germany). Total histone extraction kits were provided from Epigentek (New York, USA). Anti-acetyl histone H3K9 antibody, anti-trimethyl histone H3K27 antibody, anti-trimethyl histone H3K9 antibody and anti-histone H3 antibody were purchased from Millipore (Darmstadt, Germany), anti-rabbit IgG HRP-binding antibody were obtained from Santa Cruz (Dallas, Texas, USA). DNA methylation kits were purchased from Zymo Research (Irvine, CA, USA) and gene expression kits were obtained from Roche Life Sciences (Penzberg, Upper Bavaria, Germany). DNA, RNA isolation kits and cDNA synthesis kit were obtained from Roche Life Sciences (Penzberg, Upper Bavaria, Germany).

Cell Culture and Treatments

The human hepatocellular carcinoma (HepG2) cells were obtained from American Type Culture Collection (ATCC® HB-8065™) and maintained in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% fetal bovine serum (FBS) and penicillin-streptomycin (100 U-100 µg/mL) at 37°C in a humidified atmosphere with 5% CO_2. Subculturing was performed when the cells reached 70-80% confluence (every 2-3 days) using trypsinization.

For gene expression, DNA methylation and histone modification analysis: 1.5 x 10^6 were cultured in a 25 cm^2 culture flask for 24 h in CO_2 incubator prior to treatment. Cells were treated with BPA at the concentrations of 0.1,

1 and 10 μM and DMSO (1%) was used as control for 48 h and 96 h. At intervals of 48 h, old medium was replaced with fresh medium containing BPA. Cells were trypsinized, collected and counted by Luna cell counter (Virginia, USA) with tripan blue staining. Thus, cells were divided in three group for the extraction of genomic DNA, total RNA and histone proteins. For all concentrations, it was tested in triplicates and each test was repeated twice. The concentrations of BPA used in these experiments are similar to the concentrations used in previous studies investigating the toxicity mechanisms of BPA (Doherty et al., 2010; Qin et al., 2011; Sales et al., 2013; Feng et al., 2016; Gassman et al., 2016; Ke et al., 2016; Wang et al., 2016a).

Cytotoxicity

For cytotoxiciy assay, cells (1 x 10^4 in 100 μL medium) were seeded in 96-well plates and exposed to the BPA in the range of 0-1000 μM concentrations and DMSO (1%) as solvent control for 24 h. Then cytotoxicity tests were performed using the MTT [3-(4,5-dimethylthiazol-2-yl)-2,5 diphenyltetrazolium bromide, a tetrazole] and neutral red uptake (NRU) tests after 24 h treatment of BPA. Principle of the MTT test; yellow MTT is reduced to purple formazan in the mitochondria of living cells by the enzyme succinate dehydrogenase and it measures cell viability and proliferation (Mosmann, 1983; Alley et al., 1988). The NRU test is a cell viability test method, based on reduction in the uptake of neutral red dye into the lysosomes of cells (Borenfreund & Puerner, 1985; Repetto et al., 2008). The absorbance of formed colored solution was measured at 590 nm for MTT test and 540 nm for NRU test using a microplate spectrophotometer system (Biotek-Epoch, Winooski, USA). The cytotoxicity results were calculated as a relative percentage to the control cells and expressed as 50% of inhibitory concentration (IC_{50}) of the compound that caused 50% inhibition of the enzyme activity in the cells.

Global DNA Methylation Analysis

Genomic DNA was isolated from HepG2 cells using the High Pure PCR Template Preparation Kit (Roche Applied Science, Mannheim, Germany) according to the instructions provided by the manufacturer. DNA concentration (Optical density, OD260) and quality (OD260/280) were checked by spectrophotometric measurement.

5-mC analysis was performed using the 5-mC DNA ELISA kit (Zymo Research, Irvine, CA, USA) according to the instructions provided by the manufacturer. Briefly, 100 ng of DNA (in 100 µL of 5-mC coating buffer) in a PCR tube was denaturated at 98°C for 5 min in a thermal cycler. The denatured DNA was added to the microplate provided in the kit and incubated at 37ºC for 1 h. After washing the cells with 5-mC ELISA buffer, antibody mix was added and incubated at 37ºC for 1 h. After washing the cells, 100 µL of HRP developer was added and allowed for color development for 10-60 min. Absorbance was measured at 405 nm using a microplate spectrophotometer system (Biotek-Epoch, Winooski, USA).

5-hmC analysis was performed using the 5-hmC DNA ELISA kit (Zymo Research, Irvine, CA, USA) according to the instructions provided by the manufacturer. Briefly, 100 µl of diluted polyclonal antibody mix was added to each well and microplate was incubated at 37 ºC for 1 h. Each well was washed with ELISA buffer. DNA samples were denaturated at 98°C for 5 min in a thermocycler, then diluted with 100 µl of ELISA buffer to give a final concentration of 1 ng/µl. 100 µl of the diluted DNA samples were added to each well and incubated at 37 ºC for 1 h. Each well was washed with ELISA buffer and anti-DNA HRP antibody mix was added to each well and incubated at 37ºC for 30 min. After washing the cells, 100 µl of HRP developer was added to each well and allowed for color development for 10-60 min. Absorbance was measured at 405 nm using a microplate spectrophotometer system (Biotek-Epoch, Winooski, USA).

To quantify the percentage of 5-mC and 5-hmC, a standard curve was generated by preparing mixtures of the negative control (100 ng/µL) and positive control (100 ng/µL) standards.

Gene Specific DNA Methylation and Expression Analysis

Detection of DNA methylation in CpG islands of promoters of *p16*, *cyclin D2* and *Rassf1* genes was performed by the OneStep qMethyl Array Kit (Zymo Research, Irvine, CA, USA) according to the instructions provided by the manufacturer. Tested DNA divided into two parts as follows: Test Reaction is digested with methylation sensitive restriction enzymes (MSREs) while DNA in the reference reaction is not. Both DNA samples were amplified using real-time PCR in the presence of SYTO® 9 fluorescent dye and quantitated. Cycle threshold (Ct) values for test

and reference DNA samples will vary depending on methylation status, with large Ct differences for characteristic of unmethylated DNA. Human methylated and unmethylated DNA standards which provided with the kit also were processed along with the samples for the validation of the assay.

For gene expression analysis total RNA was isolated from HepG2 cell lines after treatment with the BPA for 48 and 96 h using an High Pure RNA Isolation Kit (Roche Life Science). Reverse transcription was performed by Transcriptor First Strand cDNA Synthesis Kit (Roche Life Science) from 1000 ng of total RNA and the combination of anchored-oligo(dT) and random hexamer primers. 5 µL of the 1/10 diluted RT-reaction were used as the template in real-time quantitavie PCR employing Light Cycler 480 Probes Master with RealTime ready Custom Single Assays (Universal ProbeLibrary Probes, Roche Life Science) which contain target specific primers for *p16*, *cyclin D2*, and *Rassf1*. The amplification reaction was performed under the following cycling conditions: 95°C for 5 min, followed by 45 cycles of 95°C for 10 s, 50°C for 15 s, 72°C for 1 s and cooling. Ct of real-time quantitavie PCR specific for *p16*, *cyclin D2*, *Rassf1* and the reference gene *β-actin* were determined. The relative expression was evaluated by the comparative Ct method.

Global Histone Modifications

Histone proteins were extracted from HepG2 cells using EpiQuik™ total histone extraction kit (Epigentek, New York, USA), according to the instructions provided by the manufacturer. Briefly, cells (10^6 cells/200 µL) were suspended in 1x pre-lysis buffer and lysed on ice for 10 min with gentle stirring. After centrifugation at 10000 rpm for 1 min at 4°C, the supernatant was removed. Then, the cell pellet was resuspended in 20 µL of lysis buffer per 10^6 cells and incubated on ice for 30 min and centrifuged at 12000 rpm for 5 min at 4°C. The supernatant fraction was transferred into a new vial and then 0.3 volumes of DTT-containing balance buffer was added to supernatant immediately. Protein concentrations of the histone proteins were measured using Bio-Rad Protein Assay II kit (Biorad, California, USA) which is based on the Bradford dye-binding method (Bradford, 1976).

Determination of histone modifications in isolated histone extracts were performed using Invitrogen western blotting system (Invitrogen, California, USA). 5 µl of lysergic acid diethylamide (LSD) sample buffer, 2 µl of sample reducing agent were added to 2 µg of protein samples, then

the volume of the mixture was completed to 20 µl with deionized water. Samples were heated to 95°C for 5 min for denaturation. Samples loaded to gel (12%) and run in 2-(N-morpholino)ethanesulfonic acid (MES) sodium dodecyl sulphate (SDS) running buffer at 165 V for 35 min. Proteins were transfered from gel to the nitrocellulose membranes with dry blotting for 7 min. Ponceau staining (Sigma-Aldrich, St Louis, Missouri, USA) is used for verify the successsful transfer and equal loading of the proteins. Nitrocellulose membrane (0.2 mm pore size) was blocked for 1 h at room temperature using 5% bovine serum albumine (5% BSA) in 0.1%TBST (50 mM Tris-HCI, pH 7.5, 150 mM NaCI, 0.1% Tween 20). Primary antibody (1:1000) was prepared by diluting in blocking buffer (5% BSA in 0.1% TBST) and the membrane incubated with primary antibody for 1.5 h at room temperature. After rinsing in 0.1% TBST solution. The membrane incubated with seconder antibody (1:5000) (anti-rabbit IgG was diluted in 1% BSA in 0.1 TBST) for 1 h at room temperature. Immunopositive bands were visualized with western blotting luminol reagent performed (Santa Cruz Biotechnology, Texas, USA) using Kodak Gel Logic System 1500 (Kodak Company, New York, USA). Band density analysis was performed using the Carestream software (Carestream, New York, USA).

Statistical Analysis

Global methylation levels (5-mC% and 5-hmC%) and cytotoxicity results were represented as mean ± standard deviation (SD). Statistical analysis was performed by ANOVA followed by Dunnett‘s multiple comparison test using “SPSS version 21.0 for Windows”, statistical program (IBM Analytics, New York, USA). P values of less than 0.05 and 0.001 were selected as the levels of significance.

Results

Cytotoxicity

Cell viability of BPA (0-1000 µM) in HepG2 cells after 24 h exposure by MTT and NRU assays was shown in Figure 1. According to MTT and NRU assays, IC_{50} values of BPA were determined as 134 and 180 µM in HepG2 cells, respectively. Figure 1 showed that 62.5, 125, 250, 500, 1000 µM treatment of BPA significantly decreased the cell viability ($p < 0.05$; $p < 0.001$) by NRU test, while 125, 250, 500, 1000 µM treatment of

BPA significantly decreased the cell viability ($p < 0.001$) by MTT test in comparison to the vehicle control group.

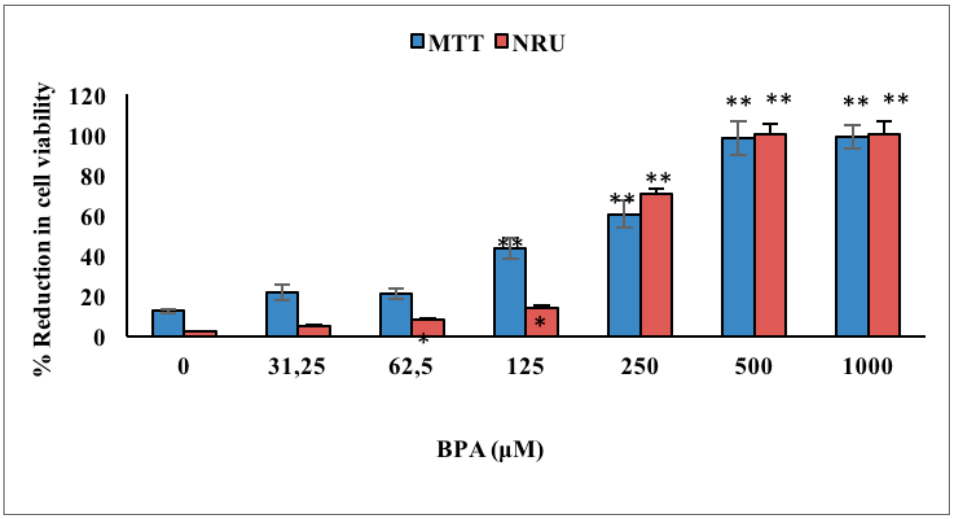

Figure 1. Effects of BPA (0–1000 µM) on cytotoxicity by MTT and NRU in HepG2 cells after 24 h exposure. Data are presented as mean ± SD. (Statistical analysis was performed by ANOVA + Dunnett post hoc test. Statistically significant changes are indicated by (*$p < 0.05$; **$p < 0.001$).

Global DNA Methylation

Figure 2 shows the effects of BPA treatment in the levels of 5-mC% in HepG2 cells after 48 h and 96 h treatment. Decreased global DNA methylation was found at the concentrations of 1 µM (3.13 fold) and 10 µM (1.75 fold) for 96 h after BPA exposures ($p<0.001$; $p<0.05$, respectively). In figure 3, it was shown that significant increase was observed in the levels of 5-hmC% at the concentration of 10 µM BPA exposure after 96 h ($p<0.001$).

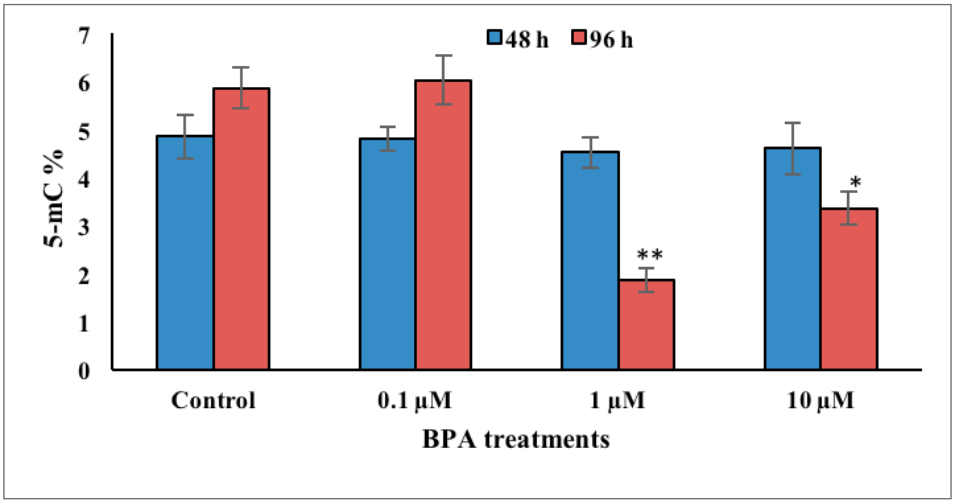

Figure 2. Effects of BPA (0.1 µM, 1 µM and 10 µM) in the levels of 5-mC% in HepG2 cells after 48 h and 96 h exposure. Data are presented as mean ± SD. Genomic DNA was extracted and 5-mC levels was detected using the 5-mC DNA ELISA kit. Statistical analysis was performed by ANOVA + Dunnett post hoc test. Statistically significant changes are indicated by (*$p < 0.05$; **$p < 0.001$).

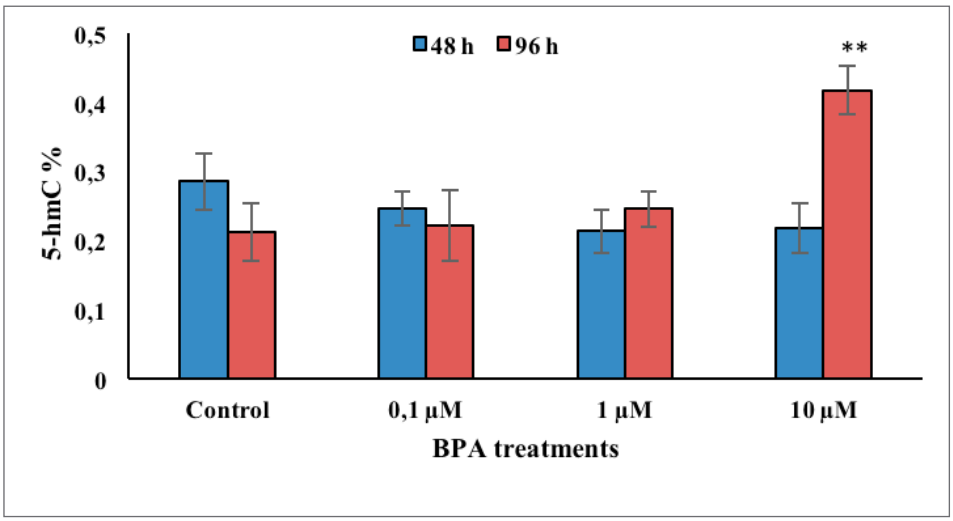

Figure 3. Effects of BPA (0.1 µM, 1 µM and 10 µM) in the levels of 5-hmC% in HepG2 cells after 48 h and 96 h exposure. Data are presented as mean ± SD. Genomic DNA was extracted and 5-hmC levels was detected using the 5-hmC DNA ELISA kit. Statistical analysis was performed by ANOVA + Dunnett post hoc test. Statistically significant changes are indicated by (**$p < 0.001$).

Gene Specific DNA Methylation and Expression of *p16*, *cyclin D2* and *Rassf1*

Effects of BPA on CpG island promoter methylation and expression profile of key cancer related genes has been analysed using real-time PCR in HepG2 cells. It has been shown that BPA caused no significant changes on both DNA methylation in the promoter regions and expressions of *p16*, *cyclin D2* and *Rassf1* genes (data not shown).

Global Histone Modifications

We investigated whether the transcriptional fluxes observed in the gene expression studies were related to absolute changes in protein levels. Western-blot experiments and digital densitometry confirmed that decrease on the global levels of H3K9me3 was observed after 0.1 and 1 µM BPA exposure for 48 h, while increase on the global levels of H3K9me3 was observed after 0.1 µM and 1 µM BPA exposure for 96 h. Hovewer, it has not been observed significantly changes for the global levels of H3K27me3 and H3K9ac (Figure 4).

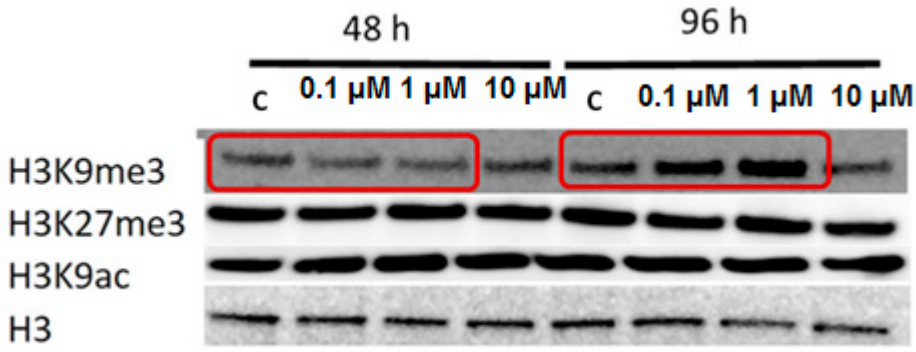

Figure 4. Representative western blots for H3K9me3, H3K27me3 and H3K9ac protein from BPA (0.1 µM, 1 µM and 10 µM) treated HepG2 cells after 48 h and 96 h exposure. H3 was used as loading control and ponceu S as an indicator of alterations of histone proteins. The experiments were performed in triplicates.

Discussion

Epigenetics includes a series of cellular mechanisms and modifying chromatin packaging (Lübbert & Jones, 2014). It has been reported that BPA can induce epigenetic changes in early life in human (Singh et al., 2012). In this study we have observed to elucidate the relationship between low-dose BPA exposure and alteration of global and gene specific DNA methylation, gene expression and histone modifications in HepG2 cell line.

IC_{50} values of BPA was determined as 134 and 180 μM in HepG2 cells for 24 h by MTT and NRU tests, respectively. Consistent with our study, Nakagawa and Tayama (2000) showed that IC_{50} of BPA was 0.25 mM in rat hepatocytes suspensions after 2 h exposure (Nakagawa & Tayama, 2000). These results are similar to the results that we have previously reported which shows IC_{50} of BPA as 148 μM in human mammary adenocarcinoma cells (MCF-7) (Senyıldız & Özden, 2015). Based on these data, in the present study for DNA methylation and histone modifications analysis we selected the concentrations of 0.1 μM, 1μM and 10 μM for BPA exposure which did not show decrease on cell viability.

Changes in global DNA methylation status are associated with X-inactivation, imprinting and the development of primordial germ cells; moreover, global DNA methylation is highly aberrant in cancer (Huang et al., 2010). We revealed significant decrease on the global levels of 5-mC% at 1 and 10 μM after 96-h BPA exposure, while significant increase were observed in the level of 5-hmC% at 10 μM after 96-h exposure. In our previous study, we demonstrated decrease on the level of global 5-mC% after BPA exposure (100 nM and 1 μM) for 48 and 96 h whereas non-significant slightly increase were observed in the levels of 5-hmC% in MCF-7 cells (Şenyıldız & Ozden, 2015).

p16, c*yclin D2* and *Rassf1* play an important role in regulation of cell cycle and tumor development via cell signaling pathways. Cyclin-dependent kinase inhibitor *p16*, a tumor suppressor protein in human, has functions in inactivating cyclin D/ cyclin-dependent kinases 4 complex (Kufe et al., 2003; Geraldes et al., 2016). Promoter hypermethylation of *p16* gene was found to be assoiated with transcriptional silencing of the gene in huıman neoplasms (Liggett & Sidransky, 1998; Geraldes et al., 2016). *Cyclin D2* is a member of the D-type cyclins, which plays a pivotal role in cell cycle regulation and differentiation and it was generally considered as a protooncogene in various tumors. Wang and collegues showed that

cyclin D2 was down-regulated in renal cell cancer and this data suggested that the function of *cyclin D2* was related to epigenetic regulation (Wang et al., 2016b). Another regulator *Rassf1* is a tumor suppressor gene which functionally involved in cell cycle control, microtubule stabilization, cellular adhesion, cell motility, and apoptosis (Agathanggelou et al., 2005; Peters et al., 2007; Araújo et al., 2016). The epigenetic downregulation of *Rassf1* is commonly associated with promoter hypermethylation (Klacz et al., 2016) involved in hepatocellular carcinoma development (Araújo et al., 2016). Araújo et al., (2016) demonstrated that hypermethylation of *Rassf1* was found to contribute to hepatocarcinogenesis and could be a valuable biomarker for early diagnosis of hepatocellular carcinoma (Araújo et al., 2016). BPA may lead to liver carcinogenesis via alterations in various signaling pathways (Weinhouse et al., 2015; 2016). Pathway analysis showed BPA exposure was related to intracellular Janus kinase (JAK)/ signal transducer and activator of transcription (STAT) and mitogen activated protein kinase (MAPK) signaling pathways that may mediate the link between early life BPA exposure and later life hepatic tumors in mice (Weinhouse et al., 2015).Therefore we decided to evaluate the promoter methylation and expression profiles of cancer related genes *p16*, *cyclin D2* and *Rassf1* in HepG2 cell line after BPA exposure using real-time PCR. In our study we did not observe significant changes both in expression and gene specific DNA methylation of *p16*, *cyclin D2* and *Rassf1* genes. However there are several studies related to alterations in the regulation of *p16* and *cyclin D2* genes in response to BPA exposure (Qin et al., 2012; Alonso-Magdalena et al., 2015). Human mammary epithelial cells (HMEC) exposed to 1 and 10 μM concentrations of BPA for a period of 1 week showed that DNA methylation levels of *p16* were increased correspondingly *p16* expression was down-regulated in a dose-dependent manner (Qin et al., 2012). In addition, DNA hypermethylation was observed in the other genes related to cancer progression such as *Brca1*, *Ccna1*, *Thbs1*, *TNFRSF10C* and *TNFRSF10D* after BPA exposure (Qin et al., 2012). In addition, Alonso-Magdalena et al. (2015) showed that p16 protein expression increased significantly in pancreatic β cells of BPA-treated pregnant mice at two different doses (10 or 100 μg/kg/day) (Alonso-Magdalena et al., 2015). Alonso-Magdalena et al. (2015) also showed decrease in protein levels of *cyclin D2* and expression of *cyclin D2* in both 10 and 100 μg/kg per day dose of BPA treatment in pancreatic

β cells of pregnant OF-1 mice from day 9 to day 16 of gestation (Alonso-Magdalena et al., 2015). It has been thought that differences between our study and other studies could result from concentrations and period of exposure of BPA and cell types.

Histone modifications are covalent post-translational modifications and they induce alterations in gene expression levels by altering chromatin structure. Histone modifications such as H3K9me3 and H3K27me3 are usually associated with gene silencing (Saksouk et al., 2015; Zeller et al., 2016). Lysine acetylation is a reversible posttranslational modification of proteins and plays a key role in regulating gene expression (Choudhary et al., 2009). Alterations in DNA methylation and histone modifications are associated with the frequency of tumor development and the several cancer type such as breast, prostate, liver (Pertega-Gomes et al., 2015; Udali et al., 2015; Spangle et al., 2016). Regarding to the studies in chemical carcinogenesis (Greathouse et al., 2012; Tabish et al., 2012; Ling et al., 2014; Liao et al., 2016) we suggest the potential role of mechanisms of histone modifications in BPA-induced toxicity. In present study, we observed decrease on the global levels of H3K9me3 after 0.1 and 1 μM of BPA exposure for 48 h, while increase on the global levels of H3K9me3 was observed after 0.1 and 1 μM of BPA exposure for 96 h. However, it has not been observed significantly changes for the global levels of H3K27me3 and H3K9ac. There are no related studies about histon modifications in HepG2 cells exposed to BPA. However, *in vitro* several studies has demonstrated that BPA caused histon modifications in different cell lines such as breast cancer, prostate and oocyte (Bhan et al., 2014; Ho et al., 2015; Wang et al., 2016a).

In conclusion, we observed that global DNA methylation and global histone modifications may be involved in BPA toxicity in HepG2 cells. Further studies might provide more detailed information for the evaluation of epigenetic alterations in BPA toxicity in cell cultures.

Acknowledgements

This work was supported in part Scientific Research Projects Coordination Unit of Istanbul University (Project numbers: ONAP-37505; NP-49518 and BYP-46016) TUBITAK (Project no. 2210-C/2014-2, Grant for Mine Senyildiz).

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