JEB
Journal of Environmental Biology

Journal Website : www.jeb.co.in
E-mail : editor@jeb.co.in
ISSN: 0254-8704 (Print)
ISSN: 2394-0379 (Online)
CODEN: JEBIDP

Bisphenol-A induced oxidative stress and apoptosis in kidney of male rats

Mai A. Elobeid* and Zeinab K. Hassan
Department of Zoology, Center for Scientific and Medical Female Colleges, King Saud University, Riyadh-11495, Saudi Arabia
*Corresponding Author's E-mail: maielobeid@gmail.com

Publication Info

Paper received:
25 March 2014

Revised received:
01 December 2014

Accepted:
25 February 2015

Abstract

Bisphenol-A (BP-A) is known to be toxic to mammalian cells. The present study investigated the ability of bisphenol-A to cause nephrotoxicity *via* aberration in the expression of apoptotic genes and oxidative stress in male rats kidney. Four groups of male Wistar rats were orally administered bisphenol-A at a dose of 0.1, 1, 10 and 50 mg kg^{-1} day^{-1} for 4 weeks. The fifth group was given water with vehicle. Significant increase in blood urea nitrogen and creatinine levels was observed in the group administered 50 mg kg^{-1} day^{-1} as compared to other groups and control. CAT activity increased insignificantly with increasing cumulative doses (10.5±0.09, 11.1±0.46, 12.6±0.34, 16.9±0.06) as compared to control (10.5±0.12). Group exposed to 50 mg BP-A (2.27±0.03*) showed significant reduction in GSH activity. *Bax* (1.7 ±0.02*) and *Bad* (2.1 ±0.01*) genes expression levels were suppressed in the group exposed to 50 mg BP-A. *BclX* gene expression was not affected by BPA in all groups. *Bcl2* gene expression was significantly up-regulated in group exposed to 50 mg BP-A (4.8 ±0.02*) as compared to control. The study showed that bisphenol-A induced nephrotoxicity through oxidative stress and by altering the apoptotic pathway involved.

Key words

Apoptosis, Bisphenol-A, Nephrotoxicity, Oxidative stress

Introduction

Bisphenol-A (BP-A) is an artificial compound that has been widely used in the production of water bottles, food containers and medical products. Recently, BP-A is reported as an endocrine disruptor that may cause obesity, diabetes and heart disease (Elobeid and Allison, 2008). BP-A has an abnormal effect of low-dose exposure depending on non-monotonic dose responses and acts like a hormone, altering cellular function at very low concentrations, with maximum safe levels of 5 mg kg^{-1} day^{-1} (Melzer *et al*., 2012). BP-A absorbed in gastrointestinal tract, may affect reproduction and development and has also seen found in human blood. It is conjugated by glucuronic acid in intestine and liver and excreted in urine as BP-A-glucuronide (Dekant and Volkel, 2008), can also disrupt immune system and may be carcinogenic (Prins *et al*., 2008).

Apoptosis is cell death to remove aberrant cells and involves two main families of proteins cysteine proteases called caspase enzymes and B-cell lymphoma 2 (*Bcl-2*) family (Piao *et al*., 2013). There are two major apoptosis signaling pathways, mitochondrial and death receptor pathways. The mitochondrial apoptotic pathway is initiated within the cell in which pro-apoptotic proteins are released from mitochondria to activate caspase proteases triggering apoptosis (Lessene *et al*., 2008). *Bcl-2* can act either as anti-apoptotic (*Bcl-xL*) mainly by inhibiting the mitochondrial pathway or pro-apoptotic (*Bcl-xS*) ((Lessene *et al*., 2008). On exposure to death signals, pro-apoptotic proteins *Bax* (*Bcl-2* associated X) and *Bak* (*Bcl-2* antagonist killer) undergo structural modifications and alter other pro-apoptotic molecules (Lalier *et al*., 2007). *Bcl-xL* binds to pro-apoptotic members of *Bcl-2* family and impairs activation of *Bax/Bak*, maintaining mitochondrial membrane integrity. Caspase 3, 8, and 9 enzymes play an effective role in apoptotic process in kidney (Scorrano and Korsmeyer, 2003).

Apoptosis can result from multiple stimuli, including free radicals *(*Piao *et al.,* 2013). Reactive oxygen species (ROS) are formed by exposure to several agents, causing oxidative damage in tissues and may lead to mitochondrial injury

consequently playing an important role in the apoptotic mechanisms (Al-Shobaili *et al*., 2013). Increased ROS production and/or decreased capacity of antioxidant defense can disrupt oxidative balance leading to activate/inactivate signaling pathways by oxidative modification of redox-active factors. ROS are removed by free radical scavengers in cells such as superoxide dismutase, catalase and reduced glutathione (Moon *et al*., 2012). The aberrant production of ROS due to contact with chemicals may result in a number of clinical disorders (Roy *et al*., 2012).

While several studies have reported the toxic effect of BP-A on reproductive, neurological and endocrine tissues in animals; data on kidney toxicity is meagre, hence this study investigated the toxic effect BP-A on kidney of male Wistar rats.

Materials and Methods

Healthy 25 male Wistar rats, two-months-old with average body weight of 300-345 g, were used in study. Rats were obtained from the Experimental Animal Unit, King Saud University, Saudi Arabia. The study was approved by research and ethical committee at King Saud University. Rats were randomly selected and caged. In a well-ventilated animal room with 12 hrs of dark/light cycle at controlled temperature and had free access to standard diet and drinking water.

Experimental design : To investigate BP-A toxicity, rats were randomly divided into five groups consisting of five rats each. Different groups of rats were administered freshly prepared BP-A (Sigma-Aldrich, USA) orally via gavage at concentrations of 0.1, 1, 10 and 50 mg kg^{-1} day^{-1} with 0.02% ethanol (vehicle) in water. Animals were administered BP-A once daily for four weeks (Hassan *et al*., 2012) as follows. Group one was administered orally 0.1 mg kg^{-1} day^{-1} of BP-A. Group two was administered daily 1 mg kg^{-1} day^{-1} of BP-A. Group three was administered daily 10 mg kg^{-1} day^{-1} of BP-A. Group four was administered daily 50 mg kg^{-1} day^{-1} of BP-A. In the fifth group, control rats were treated identically with equal volumes of 0.02% ethanol (vehicle) in water only, throughout the study. All procedures were carried out in accordance with the Guidelines for Care and Use of Laboratory Animals (NIH, 2002).

Collection of samples : 24 hrs after receiving the last dose, all animals from each group were anesthetized with ether. Blood samples were obtained from the orbital sinus of rats, using heparinized capillary tubes and collected in clean dry test tubes, then centrifuged at 3500 r.p.m for 15 min. Serum was stored at -20°C. Immediately after blood samples were collected, animals were sacrificed and dissected. Kidney were immediately removed and frozen at -80°C for enzyme assay, determination of malondialdehyde (MDA) and reduced glutathione (GSH) levels and gene expression (*Bcl2*, *Bax*, *Bad* and *Bclx*).

Biochemical analysis : Renal dysfunction was assessed by measuring rises in serum levels of blood urea nitrogen (BUN) and creatinine. Serum creatinine and urea were determined colorimetrically according to the protocol of the commercial kit (Randox Laboratories Limited, UK). Blood urea nitrogen was determined by multiplying the result of serum urea by a factor (0.467).

Renal catalase (CAT), superoxide dismutlase (SOD) activity and MDA, GSH levels were measured in kidneys by commercially available ELISA kits (Cayman Chemicals, USA). Kidneys were thawed and homogenized in ice cold phosphate buffer (50 mM pH 7.4, 0.1% triton X and 0.5 mM EDTA), then subjected to cooling centrifugation at 12,000 g for 15 min. The supernatant was used for investigating activities of antioxidant enzymes and markers of oxidative stress. Tissue homogenates for kidney from each experimental group were prepared in accordance with the protocol provided with enzyme assay kits (Cayman Chemicals, USA). The supernatant was stored in ice and used for assay. Each sample was tested in triplicate (Hassan *et al*., 2012).

Gene expression profile by Real-Time PCR in kidney : Total RNA were extracted from kidney tissue by Trizol method, according to the manufacturer's protocol. cDNA was synthesized from 1µg of total RNA by reverse transcription with SuperScript™ first-strand synthesis system kit (Invitrogen, USA), according to manufacturer's instructions. Real time PCR was done to investigate gene expression levels of studied genes: *Bcl2*, *Bax*, *Bad* and *Bcl2*-like 1 (*Bclx*). Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) gene was used as a house keeping gene.

Statistical analysis : All the experiments were performed three times independently. Differences between obtained values (mean ± SD, n = 5) were carried out by one-way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test. For statistically significant difference $p \leq 0.05$ or less was taken as a criterion.

Results and Discussion

A significant increase ($p<0.05$) was observed in BUN and creatinine levels (Table 1) in rats from the group exposed to 10 and 50 mg kg^{-1} day^{-1} BP-A doses in comparison to control and compared to the low BP-A doses did not show any significant change. The present study contradicts with Korkmaz, *et al*., (2011), who showed that vitamin C coadministration with BP-A did not produce significant increase in BUN concentration in BP-A group. Renal dysfunction is usually assessed by an elevated BUN and creatinine level indicating that the highest dose of 50 mg of BP-A may have led to that. BUN and creatinine levels were usually not raised above the normal range until 60% of total kidney function was lost. Creatinine has been found to be a fairly reliable indicator of kidney function; hence, elevated creatinine

Table 1: Kidney function in rats exposed to cumulative doses of BPA

Groups (mg kg^{-1} day^{-1})	BUN (mg dl^{-1})	Creatinine (mg dl^{-1})
Control	25.4± 0.378	1.60±0.02
0.1 mg kg^{-1} day^{-1} BPA	25.5±0.333	1.61±0.03
1 mg kg^{-1} day^{-1} BPA	26.5±1.022	1.66±0.06
10 mg kg^{-1} day^{-1} BPA	31.2±0.778*	1.83±0.04*
50 mg kg^{-1} day^{-1} BPA	45.5±0.333*	2.60±0.07*

Data is presented as the mean of concentrations levels ± SD. * indicated significant change from control p<0.05

Table 2 : Effect of different doses of BPA administration on CAT, GSH, MDA and SOD activity in kidney tissue

Groups	CAT (µmol min^{-1} g^{-1} tissue)	GSH (µmol min^{-1} g^{-1} Tissue)	MDA (µM g^{-1} tissue)	SOD (U ml^{-1} g^{-1} tissue)
Control	10.5±0.12	5.47±0.09	13.4±0.03	626.7±3.5
0.1 mg Kg^{-1} day^{-1} BPA	10.5±0.09	5.57±0.03	13.6±0.03	628.3±4.7
1 mg Kg^{-1} day^{-1} BPA	11.1±0.46	5.03±0.15	13.7±0.15	620.7±5.2
10 mg Kg^{-1} day^{-1} BPA	12.6±0.34	4.81±0.08	15.1±0.47	611.3±0.7
50 mg Kg^{-1} day^{-1} BPA	16.9±0.06	2.27±0.03*	32.6±0.20*	513.7±4.7*

Data is presented as mean of concentrations ± SD. * indicated significant change from control p < 0.05

Table 3 : Effect of BPA concentrations on apoptosis gene expression level in kidney tissue

Groups	Bax	Bad	BclX	Bcl2
Control	3.4±0.43	3.1±0.2	2.4±0.21	2.9±0.4
0.1 mg Kg^{-1} day^{-1} BPA	3.1 ±0.1	2.9±0.1	2.2±0.08	2.9±0.1
1 mg Kg^{-1} day^{-1} BPA	2.9±0.1	2.7±0.03	2.3±0.0	2.2±0.04
10 mg Kg^{-1} day^{-1} BPA	2.5±0.03	2.5±0.02	2.5±0.03	2.8±0.03
50 mg Kg^{-1} day^{-1} BPA	1.7±0.02*	2.1±0.01*	2.3±0.01	4.8±0.02*

Data is presented as mean of fold expression ± SD. * indicated significant change from control p < 0.05

level signifies impaired kidney function or kidney disease.

ROS are generated under normal cellular conditions and are detoxified by major scavenger enzymes such as glutathione based enzymes, SOD and CAT (Juranek *et al*., 2013). Groups exposed to BP-A showed an insignificant increase in CAT activity with an increase in the cumulative BP-A doses in CAT levels as compared to control group (Table 2). A significant reduction in GSH level and SOD activity ($p < 0.05$) was observed in the group exposed to 50 mg BP-A as compared to other groups. A recent study has showed that BP-A causes hepatic toxicity and pancreatic dysfunction via impairing mitochondrial function and alterations in expression of genes and metabolism (Lin *et al*., 2013). Oxidative stress plays an important role in kidney pathogenesis (Parlakpinar *et al*., 2005). The aberrant level of GSH and SOD activities in kidneys in the BP-A group exposed to 50 mg showed that BP-A may cause nephrotoxicity via free radical generation. GSH plays an important role in detoxification of xenobiotic compounds and in antioxidation of reactive oxygen species and free radicals. Similarly, the aberrant levels of GSH were observed with oxidative stress (Zou *et al*., 2012). Excessive ROS production by BP-A can could antioxidant imbalance and could lead to lipid peroxidation and antioxidant depletion (Lam *et al*., 2011).

Lipid peroxidation was assessed as an index of oxidative stress. The present study indicated that lipid peroxidation in terms of MDA level in kidneys was significantly increased among rats administered 50 mg of BP-A. This result is in agreement with the findings of previous study which had demonstrated the involvement of oxidative stress and lipid peroxidation in BP-A induced liver toxicity (Lam *et al*., 2011). Elevation in MDA and CAT activity was accompanied by a significant suppression in level GSH and SOD activity in kidney of rats with high dose of BP-A showed an oxidative stress condition. In line with this, a recent study reported that BP-A-induced damage was associated with oxidative stress (Hassan *et al*., 2012).

In order to investigate the relation between BP-A concentration and cell death pathways, pro- and anti-apoptotic genes were studied in the present study. The *Bax* and *Bad* genes expression levels were significantly suppressed in high BP-A concentration group of rats, which may be indicative of an apotic pathway (Table 3). This is in agreement with the previous study of Lin *et al.* (2013) that found BPA suppressed cell viability and disturbed insulin secretion in a dose-dependent manner. However, gene expression level of *BclX* was not affected by BP-A.

The *Bcl2* gene was significantly up-regulated in 50 mg kg^{-1} day^{-1} BP-A group. It is well known that *Bax* to *Bcl-2* ratio determines the fate of cells. *Bax* and *Bcl-2* can either homo- or heterodimerize. Heterodimerization between proapoptotic *Bax* and antiapoptotic *Bcl-2* may negate the function of either protein. An excess of *Bcl-2* homodimers favors cell survival, whereas that of *Bax* homodimers favors cell death. Thus, *Bax* to *Bcl-2* ratio serve as a rheostat to determine the susceptibility to apoptosis. In the current study, in kidney, the anti-apoptotic factor *Bclx* mRNA were normally expressed in all the experimental groups with suppression of pro-apoptotic factor, *Bax* and *Bad* after BP-A administration. Induction of apoptosis by *Bcl-2* includes participation of oxidative stress.

Endocrine disrupting effects of BP-A is well recognized. Various doses of BP-A inflicted oxidative stress by affecting the status in kidney. The findings of the present study demonstrated that high dose of BPA 50 mg kg^{-1} may damage kidney, affecting oxidant/antioxidant balance, while low dose of 10 mg kg^{-1} could also cause damage and must be taken in consideration. Furthermore, BP-A could cause developmental toxicity through

anti-proliferation and pro-apoptosis.

Acknowledgment

The authors extend their appreciation to the Deanship of Scientific Research at King Saud University for funding the work through the research group project no. RGP-VPP-033.

References

Al-Shobaili, H., A. Al-Robaee, A. Alzolibani and Z. Rasheed: Immunological studies of reactive oxygen species damaged catalase in patients with systemic lupus erythematosus: correlation with disease activity index. *Immunol. Invest.*, **42**, 191-203 (2013).

Dekant, W. and W. Volkel: Human exposure to bisphenol A by biomonitoring: methods, results and assessment of environmental exposures. *Toxicol. Appl. Pharmacol.*, **228**, 114-134 **(**2008).

Elobeid, M. and D. Allison: Putative endocrine disruptors and obesity: A Review. *Curr. Opin. Endocrinol. Diab.*, **15**, 403-408 (2008).

Hassan, Z., M. Elobeid, P. Virk, S. Omer, M. ElAmin, M. Daghestani and E. AlOlayan: Bisphenol A induces hepatotoxicity through oxidative stress in rat model. *Oxid. Med. Cell. Longev.*, **2012,** 194829 (2012).

Juranek, I., D. Nikitovic, D. Kouretas, A. Hayes and M. Tsatsakis: Biological importance of reactive oxygen species in relation to difficulties of treating pathologies involving oxidative stress by exogenous antioxidants. *Food Chem. Toxicol.*, **61**, 240-247 (2013).

Korkmaz, A., A. Mu, K. Du and B. Nurhayat: Vitamin C Co administration augments bisphenol a, nonylphenol, and octylphenol induced oxidative damage on kidney of rats. *Environ. Toxicol.*, **26**, 325-337 (2011).

Lalier, L., P. Cartron, P. Juin, S. Nedelkina, S. Manon, B. Bechinger and F. Vallette: Bax activation and mitochondrial insertion during apoptosis. *Apoptosis*, **12**, 887-896 (2007).

Lam, S.H., M. Haing, X. Zhang, C. Yan, Z. Duan, L. Zhu, C. Ung, S. Mathavan, C. Ong and Z. Gong: Toxicogenomic and phenotypic analyses of bisphenol-A early-life exposure toxicity in zebrafish. *PloS One*, **6**, e28273 (2011).

Lessene, G., Czabotar, P. and P. Colman: BCL-2 family antagonists for cancer therapy. *Nat. Rev. Drug. Discov.*, **7**, 989-1000 (2008).

Lin, Y., X. Sun, L. Qiu, J. Wei, Q. Huang, C. Fang, T. Ye, M. Kang, H. Shen and S. Dong: Exposure to bisphenol A induces dysfunction of insulin secretion and apoptosis through the damage of mitochondria in rat insulinoma (INS-1) cells. *Cell Death Dis.*, **4**, e460. (2013).

Melzer, D., P. Gates, N. Osborne, W. Henley, R. Cipelli, A. Young, C. Money, P. McCormack, P. Schofield, D. Mosedale, D. Grainger and T. Galloway: Urinary bisphenol a concentration and angiography-defined coronary artery stenosis. *PloS One*, **7**, e43378 (2012).

Moon, H., W. Ko, S. Han, D. Kim, Y. Hwang, H. Park and I. Kwon: Antioxidants, like coenzyme Q10, selenite, and curcumin, inhibited osteoclast differentiation by suppressing reactive oxygen species generation. *Biochem. Biophys. Res. Commun.*, **418**, 247-253 (2012).

National Institute of Health, USA. Public health service policy on humane care and use of laboratory animals, (2002).

Piao, J., Z. Cui, Y. Furusawa, K. Ahmed, M. Rehman, Y. Tabuchi, M. Kadowaki and T. Kondo: The molecular mechanisms and gene expression profiling for shikonin-induced apoptotic and necroptotic cell death in U937 cells. *Chemi. Biol. Interact.*, **205**, 119-127 (2013).

Prins, G., W. Tang, J. Belmonte and S. Ho: Developmental exposure to bisphenol A increases prostate cancer susceptibility in adult rats: epigenetic mode of action is implicated. *Fertil. Steril.*, **89**, e41 (2008).

Parlakpinar, H., S. Tasdemir, A. Polat, A. Bay-Karabulut, N. Vardi, M. Ucar and A. Acet: Protective role of caffeic acid phenethyl ester (cape) on gentamicin-induced acute renal toxicity in rats. *Toxicology*, **207**, 169-77 (2005).

Roy, N., S. Bagchi and P. Raychaudhuri: Damaged DNA binding protein 2 in reactive oxygen species (ROS) regulation and premature senescence. *Int. J. Mol. Sci.*, **13**, 11012-26 (2012).

Scorrano, L. and J. Korsmeyer: Mechanisms of cytochrome c release by proapoptotic BCL-2 family members. *Biochem. Biophys. Res. Commun.*, **304**, 437-444 (2003).

Zou, X., Z. Feng, Y. Li, Y. Wang, K. Wertz, P. Weber, Y. Fu and J. Liu: Stimulation of GSH synthesis to prevent oxidative stress-induced apoptosis by hydroxytyrosol in human retinal pigment epithelial cells: activation of Nrf2 and JNK-p62/SQSTM1 pathways. *J. Nutr. Biochem.*, **23**, 994-1006 (2012).