

Bisphenol-A Induces Oxidative Damage in the Liver of Chicken Embryos

Soraya Gharibi,¹ Parisa Sadighara,² Tahereh Mohajerfar,¹ Ramin Mazaherinezhad-Fard,³ Tahereh Farkhondeh*⁴

1. Academic Center for Education, Culture and Research, University of Tehran, Tehran, Iran
2. Department of Environmental Health, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran
3. Rastegar Reference Laboratory, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
4. Department of Environmental Health, Food Safety Division, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran

Article information	Abstract
<p>Article history: Received: 5 Jan 2012 Accepted: 24 Oct 2012 Available online: 12 Mar 2013 ZJRMS 2013; 15(10): 12-15</p> <p>Keywords: Bisphenol A Chicken Embryos developmental Toxicity</p> <p>*Corresponding author at: Department of Environmental Health, Food Safety Division, Faculty of Public Health, Tehran University of Medical Sciences, Tehran, Iran E-mail: Farkhondeh2324@gmail.com</p>	<p>Background: Oxidative stress mechanisms are involved in the embryotoxicity. The objective of this study was to assess hepatotoxicity of bisphenol-A (BPA) in chicken embryos.</p> <p>Materials and Methods: Fertile eggs were randomly divided into 4 groups; three experimental and one control groups, (N=15, for each group). Embryos were administered 50, 100 and 200 PPM BPA, and incubated for 48 h at 37°C with a relative humidity of 63%. The experiment was terminated on day 20 of incubation. Then, the embryos were decapitated and livers of embryos were collected for biochemical analysis. The total antioxidant capacity, malondialdehyde (MDA), glutathione (GSH), carotenoid and total protein levels were measured by spectrophotometer.</p> <p>Results: The result of the present study indicated that the levels of total antioxidant in the livers of embryos exposed to 200 PPM BPA were higher than control and other groups, as well as the levels of MDA compared to control group ($p<0.05$). The levels of GSH, total carotenoids and total protein were higher in all groups exposed to BPA than control group ($p<0.05$). In addition, protein concentration in 200 PPM group was higher than of other groups ($p<0.001$).</p> <p>Conclusion: So far, BPA may lead to induce toxic response of oxidative system in liver throughout embryonic period.</p> <p>Copyright © 2013 Zahedan University of Medical Sciences. All rights reserved.</p>

Introduction

Bisphenol A (BPA: 2, 2-bis-(4-hydroxyphenyl)propane) is an organic compound composed of two phenol rings connected by a methyl bridge, with two methyl functional groups attached to the bridge, and widely used to make polycarbonate polymers and epoxy resins, along with other materials used to make plastics. BPA exhibits estrogenic hormone-like properties which can disrupt endocrine system and may lead to negative health effects [1]. It seems that the early stage of mammalian life is the most sensitive period of the live to BPA effects [2].

Due to the penetration the blood-brain and the placental barriers, this substance is a serious threat to developing nervous system in fetus, infant and young children. According to the pervious studies, BPA can cause permanent brain damage in the early stage of mammalian life. For a long time, small changes in the levels of estrogenic hormones have been considered as the main reason for damage brain caused by BPA [3]. However Oka et al. demonstrated that the effects of BPA on the developing nervous system of *Xenopus laevis* are independent of its estrogenic activities [4]. Recently, this was suggested that BPA disrupt the balance of the oxidant and anti-oxidant system. BPA may show its toxicity by increasing hydrogen peroxide in mice [5].

These authors suggested that exposure to BPA throughout embryonic/fetal life and during infancy induces tissue oxidative stress response, ultimately leading to underdevelopment of the brain, kidney and testis in mice [6].

BPA also decreases the levels of the antioxidant enzymes in the liver of male rats [7]. However, the exact mechanism of developmental toxicity of BPA has not been understood. Therefore, this study was designed to investigate the effects of BPA on oxidative stress parameters in the liver of chicken embryos. Total antioxidant capacity, tripeptide glutathione (GSH) and carotenoids (tetraterpenoids), malondialdehyde (MDA levels, index of lipid peroxidation), were measured as indices of modification in the balance between oxidant/antioxidant systems.

Materials and Methods

All materials were purchased from Sigma Company, USA. Protocol of chicken embryotoxicity study has gained acceptance by several regulating agencies [8]. It is a sensitive, inexpensive and rapid toxicity test, providing information on embryonic lethality, teratogenicity, growth retardation, metabolism as well as systemic toxicity and

immunopathological effects of chemical substance [9]. Sixty of fertile leghorn eggs were obtained on the second day from Iran farm, Karj, Iran, Infertile and damaged eggs were discarded. Following sterilization with ethanol, fertile and healthy eggs randomly divided into 4 groups; three experimental groups: 50 PPM (exposed to 50 PPM BPA), 100 PPM (exposed to 100 PPM BPA), 200 PPM (exposed to 200 PPM BPA) and one control group, (N=15, for each group). On day 4 the egg shell was opened to obtain access to the air cell, where all the test substance was pipetted directly onto the inner shell membrane. BPA powder was dissolved in 0.5 ml of extra virgin olive oil and three doses of BPA in 50, 100 and 200 ppm were injected into egg yolks while the control group only received pure olive oil. The dose was confirmed according to pervious study [10]. The holes in the egg shells were sealed with paraffin, and the eggs were placed horizontally in the incubator at 37°C with a relative humidity of 63%. The experiment was terminated on day 20 of incubation. Then, the embryos were decapitated and livers of embryos were collected for biochemical analysis. The isolated whole liver tissue was homogenized with 10 times (w/v) sodium phosphate buffer. The homogenate was centrifuged at 3000 rpm for 15 min, and the supernatant was used for estimation of biochemical indices.

Antioxidant capacity of serum was determined by measuring the ability of serum to reduce Fe^{3+} to Fe^{2+} . The complex between Fe^{2+} and TPTZ gives a blue color with absorbance at 593 nm [11]. Malondialdehyde levels (MDA, index of lipid peroxidation), were measured [12]. MDA reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substance (TBARS) to produce a red colored complex. The absorbance of the supernatant was measured at 532 nm by spectrophotometer. Total SH groups of serum was measured spectrophotometrically at 412 nm using DTNB as the reagent [13].

Carotenoids belong to the tetraterpenoids category and most of them have antioxidant activity. The total amounts of carotenoids were determined by beta-carotene standard curve and by spectrophotometric method at 470 nm. The total carotenoid content of the samples was calculated on the basis of the standard curve of beta-carotene [14]. Protein content was measured by Bradford method. Bovine serum albumin was used as standard [15]. Data were analyzed using ANOVA-one way by SPSS-16 followed by Tukey-Kramer post-hoc test for multiple comparisons. Kolmogorov Smirnov tests showed that

these data were normally distributed. The evaluation was made by the comparison of groups. The results were presented as means \pm SEM and $p < 0.05$ was considered significant.

Results

The results were illustrated in table 1. There was no significant difference between the levels of antioxidant in livers of 50 and 100 PPM groups compared to the control group, but in high dose group (200 PPM) antioxidant level was higher than control and other groups ($p < 0.001$ for 200 PPM vs. control group and $p < 0.05$ for 200 PPM vs. 50 and 100 PPM groups).

The levels of MDA in liver were higher in 200 PPM group than control group ($p < 0.05$). GSH and total carotenoids of the liver in experimental groups was significantly higher than the control group ($p < 0.05$ for 50 and 100 PPM vs. C group and $p < 0.01$ for 200 PPM vs. C group). Protein concentration in 100 and 200 PPM groups was significantly higher than controls ($p < 0.001$ for 100 and 200 PPM vs. C group and $p < 0.01$ for 100 PPM vs. C group). In addition, protein concentrations in 200 PPM group was significantly higher than of other groups ($p < 0.001$).

Discussion

The result of the present study indicated that the levels of antioxidant in the livers of 200 PPM group were higher than control and other groups, as well as the levels of MDA compared to control group. The levels of GSH, total carotenoids and total protein in all experimental groups were higher than the control group. In addition, protein concentration in 200 PPM group was higher than of other groups.

Many xenobiotics may lead to induce teratogenesis via bioactivation of embryonic cytochromes P450, prostaglandin H synthase (PHS) and reactive oxygen species (ROS). Free radicals are cytotoxic agents that lead to significant oxidative damage after causing covalently bind to oxidize cellular macromolecules such as DNA, protein and lipid lead to cell death [16]. There is evidence that several teratogen agents affect the developing embryo by increasing oxidative stress and result in severe embryonic damages due to the embryo's relatively weak antioxidant defense especially at early stages of organogenesis [17].

Table 1. Oxidative and antioxidant parameters in chicken embryos were exposed to 50, 100 and 200 PPM of BAP (for each group, N=15)

Parameters	Control	50 PPM	100 PPM	200 PPM
Total antioxidant activity (FRAP)	1.48 \pm 0.14	2.08 \pm 0.13	2.11 \pm 0.16	2.76 \pm 0.23 a***,b*,c*
MDA	0.217 \pm 0.03	0.43 \pm 0.08	0.39 \pm 0.03	0.53 \pm 0.10 a*
Intracellular GSH	0.07 \pm 0.01	0.12 \pm 0.01a*	0.12 \pm 0.00 a*	0.13 \pm 0.02 a**
Total carotenoids	0.85 \pm 0.05	1.02 \pm 0.01 a*	1.05 \pm 0.02 a*	1.13 \pm 0.03 a**
Total protein	4.26 \pm 0.37	6.76 \pm 0.51a***	6.03 \pm 0.18 a**	9.26 \pm 0.16 a***,b***,c***

Values are presented as Mean \pm SEM.

Statistical significance for difference the between the data of C vs. 50, 100 and 200 PPM groups; a*: $p < 0.05$, a***: $p < 0.001$.

Statistical significance for difference the between the data of 50 PPM vs. 200 PPM groups; b*: $p < 0.05$, b***: $p < 0.001$.

Statistical significance for difference the between the data of 100 PPM vs. 200 PPM groups; c*: $p < 0.05$, c***: $p < 0.001$.

Authors demonstrated that in embryo culture, addition of superoxide dismutase (SOD) or catalase to the medium blocked the oxidative lesions and embryotoxicity initiated by phenytoin [16]. BPA can affect the organ development, but there are not sufficient studies on the impact of BPA exposure during gestation and lactation and specific mechanisms.

This study investigated whether BPA causes hepatotoxicity by induction of oxidant/antioxidant imbalance in chicken embryo liver. Oxidative stress was focused as mechanism by which BPA leads to embryotoxicity because BPA injection has been shown to enhance oxidative stress and lipid peroxidation promoting apoptosis in several organs and disrupt cell-to-cell communication. However, this is an interesting subject because BPA can act both as a pro-oxidant induces the formation of reactive oxygen species and an antioxidant [18]. Similar to our results, Hassan et al demonstrated that 50 mg/kg of BPA generate ROS, but reduce antioxidant content and activity (glutathione, superoxide dismutase, glutathione peroxidase, glutathione-S-transferase, glutathione reductase and catalase) and led to hepatotoxicity [19]. The results of another study also revealed decrease of oxidative damage in liver and kidney of mice after exposure to BPA by potent antioxidants such as quercetin. They also showed that exposure to BPA caused significant reduction in the activities of catalase, superoxide dismutase, glutathione peroxidase, glutathione reductase and glutathione-S-transferase as well as in the levels of glutathione and total ascorbic acid contents; however, significant increase was found in the levels of malondialdehyde (MDA) [20]. In addition, hepatic necrosis and congestion were observed in livers of rats exposed to BPA because of ROS induction and disruption the balance between

ROS and antioxidant defenses system [21]. Oxidative stress has been proven to be related to BPA toxicity in animal models for years. Kabuto et al. revealed that injection of BPA induces overproduction of hydrogen peroxide in the mouse organs. Hydrogen peroxide is easily converted to hydroxy radicals. Their results have also revealed decrease in the levels of GSH and increase in the levels of oxidized glutathione (GSSG) by hydroxy radicals [5]. Hepatotoxicity of BPA was also seen after administration doses below the NOAEL that is associated with an increase in oxidative stress and inflammation [22]. Results of the present study indicated that BPA increased the levels of MDA, however, the levels of antioxidant also increase in experimental groups. Therefore embryo protective pathways against oxidative stress were able to decrease the risk of free radical in these concentrations of BPA [23]. Collectively, our experience shows that BPA may lead to induce toxic response of oxidative system throughout embryonic period.

Acknowledgements

This study was financially supported by the Academic Center for Education, Culture, and research (ACECR) of Tehran University. The authors appreciate Dr. Parisa Sadighara for supporting this study.

Authors' Contributions

All authors had equal role in design, work, statistical analysis and manuscript writing.

Conflict of Interest

The authors declare no conflict of interest.

Funding/Support

University of Tehran.

References

1. Staples CA, Dome PB, Klecka GM, et al. A review of the environmental fate, effects, and exposures of bisphenol A. *Chemosphere* 1998; 36(10): 2149-73.
2. vom Saal FS, Akingbemi BT, Belcher SM, et al. Chapel Hill bisphenol A expert panel consensus statement: Integration of mechanisms, effects in animals and potential to impact human health at current levels of exposure. *Reprod Toxicol* 2007; 24(2): 131-8.
3. Nicolas K, Emily JC, Emmanuel S, et al. Developmental and metabolic brain alterations in rats exposed to bisphenol A during gestation and lactation. *Int J Dev Neurosci* 2011; 29(1): 37-43.
4. Oka T, Adati N, Shinkai SK, et al. Bisphenol A induces apoptosis in central neural cells during early development of *Xenopus laevis*. *Biochem Biophys Res Commun* 2003; 312(4): 877-82.
5. Kabuto H, Hasuiki S, Minagawa N and Shishibori T. Effects of bisphenol A on the metabolisms of active oxygen species in mouse tissues. *Environ Res* 2003; 93(1): 31-35
6. Kabuto H, Amakawa M, Shishibori T. Exposure to bisphenol A during embryonic/fetal life and infancy increases oxidative injury and causes underdevelopment of the brain and testis in mice. *Life Sci* 2004; 74(24): 2931-2940.
7. Bindhumol V, Chitra KC, Mathur PP. Bisphenol A induces reactive oxygen species generation in the liver of male rats. *Toxicology* 2003; 188(2-3): 117-124.
8. Wolf T, Luepke NP. Formation of micronuclei in incubated hen's eggs as a measure of genotoxicity. *Mutat Res Genet Toxicol Environ Mutagen* 1997; 394(1-3): 163-175.
9. Kemper FH, Luepke NP. Toxicity testing by the hen's egg test (HET). *Food Chem Toxicol* 1986; 24: 647-648.
10. Kenichi S, Azusa N, Eri K and Mari W. Bisphenol A and estrone-induced developmental effects in early chick embryos. *Environ Toxicol* 2011; 27(1): 58-62.
11. Benzie IF, Strain JJ. The ferric reducing ability of plasma (FRAP) as a measure of "antioxidant power": The FRAP assay. *Anal Biochem* 1996; 239(1): 70-6.
12. Halliwell B, Whiteman M. Measuring reactive species and oxidative damage in vivo and in cell culture: How should you do it and what do the results mean? *Br J Pharmacol* 2004; 142(2): 231-55.
13. Gibson XA, Shartava A, McIntyre J, et al. The efficacy of reducing agents or antioxidants in blocking the

- formation of dense cells and irreversibly sickled cells in vitro. *Blood* 1998; 91(11): 4373-4378.
14. Thaipong K, Boonprakob U, Cisneros-Zevallos L and Byrne DH. Hydrophilic and lipophilic antioxidant activities of guava fruits. *Southeast Asian J Trop Med Public Health* 2005; 36 (4): 254-7.
 15. Lowry OH, Rosenbrough NJ, Farr AL and Randall RJ. Protein measurement with the folin phenol reagent. *J Biol Chem* 1951; 193: 265-75.
 16. Wells PG, Kim PM, Laposa RR, et al. Oxidative damage in chemical teratogenesis. *Mutat Res Genet Toxicol Environ Mutagen* 1997; 396(1-2): 65-78.
 17. Ornoy A. Embryonic oxidative stress as a mechanism of teratogenesis with special emphasis on diabetic embryopathy. *Reprod Toxicol* 2007; 24(1): 31-41.
 18. Jemec A, Tisler T, Erjavec B and Pintar A. Antioxidant responses and whole-organism changes in *Daphnia magna* acutely and chronically exposed to endocrine disruptor bisphenol A. *Ecotoxicol Environ Saf* 2012; 12: 147-6513.
 19. Hassan ZK, Elobeid MA, Virk P, et al. Bisphenol A induces hepatotoxicity through oxidative stress in rat model. *Oxid Med Cell Longev* 2012; 2012: 1-6.
 20. Sangai NP, Verma RJ, Trivedi MH. Testing the efficacy of quercetin in mitigating bisphenol A toxicity in liver and kidney of mice. *Toxicol Ind Health* 2012. [Epub ahead of print].
 21. Korkmaz A, Ahabab MA, Kolankaya D and Barlas N. Influence of vitamin C on bisphenol A nonylphenol and octylphenol induced oxidative damages in liver of male rats. *Food Chem Toxicol* 2010; 48(10): 2865-71.
 22. Moon MK, Kim MJ, Jung IK, et al. Bisphenol A impairs mitochondrial function in the liver at doses below the no observed adverse effect level. *J Korean Med Sci* 2012; 27(6): 644-52.
 23. Sadighara P, Salar-Amoli J, Ashrafihelan J, et al. The developmental toxicity of cottonseed extraction on chicken embryo. *Rev Bras Farmacogn* 2011; 21(3): 560-563.