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Catalase Deficiency Worsens Toxic Effects Of Bisphenol A (BPA) On Ovarian Steroidogenesis

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Abstract

Among the various proposed etiological factors responsible for female infertility, environmental pollutants accounts for the majority of infertility cases around the world. Bisphenol A (BPA) is one of such pollutants to have strong association with variety of disorders of the female reproductive system. Several studies have linked BPA to redox homeostasis through imbalance of reactive oxygen species (ROS) generation and depletion in various tissues and cell types including ovary. This crucial redox balance is maintained by antioxidant enzymes including superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx). Among these, catalase is one of such antioxidant enzymes which has highest turnover number of all such enzymes and thereby protecting the tissue from injury and damage to maintain homeostasis. But the specific effect of catalase in the BPA mediated effect on ovarian steroidogenesis is still pending. In this backdrop, the specific aim of this study was to investigate the possible outcome in the ovarian steroidogenesis in rats exposed to BPA in presence or absence of 3-Amino-1,2,4-triazole (ATZ) (catalase inhibitor). Female Wistar rats aged 8 weeks were administered BPA (25 mg/kg BW/day for 9 days, intraperitonially) with or without the pretreatment of the catalase- specific inhibitor 3-amino-1,2,4-triazole (ATZ; 1 g/kg BW/day for 5 days, intraperitoneally). Serum level of LH, FSH, estrogen and testosterone were measured using ELISA kits. Results revealed that BPA alters the ovarian steroidogenesis as evidenced by altered level of gonadotropins and sex steroids. Catalase deficiency further worsens these BPA induced effect on ovary. In conclusion, catalase deficiency mounted additional adverse condition on BPA toxic effects on the activity of theca-interstitial cells and ovarian steroidogenesis and targeting of catalase may be of therapeutic importance in the adverse effect of BPA on ovary.

Keywords: BPA, reactive oxygen species, ovary, LH, FSH, estrogen

Introduction

In our everyday experiences, individuals encounter numerous detrimental substances that are commonly employed regularly. One primary goal of life sciences and biomedical research is to recognize the impacts of

these harmful elements and their influence on human health. Bisphenol A, commonly referred to as BPA, stands out among the many environmental pollutants known for its adverse effects. This harmful chemical compound is produced globally in substantial quantities, reaching approximately 3.6 million tons annually. This compound is utilized in the production of polycarbonate plastics and epoxy resins, commonly used for crafting containers to store food and beverages. Over time, the continuous use of such containers can result in the leaching of BPA, contaminating the stored food and drinks, and subsequently accumulating in the human body, increasing susceptibility to further harm. The direct release of BPA into the environment occurs during manufacturing and processing activities, leading to its dissemination into the air, water, and soil. Recent research underscores that in everyday exposure to BPA, it functions as an endocrine-disrupting compound (EDCs), even at low doses (Fenichel *et al.,* 2013). Consequently, exposure to such low levels of EDCs may raise concerns, as they can interfere with numerous metabolic processes, leading to widespread damage to body tissues and the onset of cardiovascular diseases. Recent research has demonstrated that exposure to BPA in the past year is associated with negative health outcomes during perinatal, childhood, and adulthood stages, including reproductive and developmental effects, metabolic diseases, and other health issues. Recent investigations have revealed that both human and wildlife populations are exposed to varying levels of BPA, with the capacity to adversely affect reproductive, developmental, and metabolic endpoints across diverse wildlife species and laboratory animal studies (Calafat *et al.,* 2008). Studies suggest that BPA acts as an estrogen mimetic compound, contributing to a range of health impacts, including the development of prostate and breast cancer (Prins *et al.,* 2008). The adverse effects of BPA are predominantly linked to its estrogenic activity; however, BPA also induces effects such as dysregulation of inflammatory cytokines and mitochondrial-mediated apoptosis in hepatic tissue.

Furthermore, there is compelling evidence indicating a significant correlation between urine concentration of BPA and various health disorders, including cardiovascular issues, type 2 diabetes, endometrial hyperplasia, recurrent miscarriages, polycystic ovarian syndrome, and neurobehavioral problems such as attention deficit hyperactivity disorders and developmental effects in humans. Evidence indicates that BPA can disrupt the endocrine function of the hypothalamic-pituitary axis by altering the secretion of gonadotropin-releasing hormones (GnRH) in the hypothalamus and promoting pituitary proliferation. These actions have consequences for puberty, ovulation, and may potentially lead to infertility. Furthermore, BPA adversely affects various reproductive organs such as the ovaries and uterus. Exposure to BPA hampers the structure and functions of the female reproductive system at different stages of the life cycle, potentially contributing to infertility. Numerous studies have demonstrated that exposure to BPA results in both morphological and functional changes in the female reproductive system, particularly affecting the ovaries (Caserta *et al.,* 2014).

BPA exhibits various effects on the ovaries, including an association with follicle loss. Polycystic ovary syndrome (PCOS), the most common endocrinopathy among women of reproductive age, has been linked to BPA exposure. Recent findings indicate that women with PCOS have significantly higher levels of BPA compared to those without PCOS (Huo et al., 2015). Ovaries exposed to BPA exhibit a reduction in size and display signs of ovarian cysts, resembling those observed in many women with PCOS. Additionally, there is a higher prevalence of degenerating eggs compared to relatively fewer healthy, developing ones. These conditions contribute to a failure to ovulate or conceive, leading to impaired fertility in affected women.

Conversely, catalase is a ubiquitous enzyme present in nearly all oxygen-exposed living organisms, including bacteria, plants, and animals. Its primary function is to catalyze the breakdown of hydrogen peroxide into water and oxygen. This enzyme plays a crucial role in safeguarding cells from oxidative damage caused by reactive oxygen species (ROS). Notably, catalase boasts one of the highest turnover numbers among all enzymes, with a single catalase molecule capable of converting millions of hydrogen peroxide molecules into water and oxygen per second. This rapid action serves to protect tissues, preventing injury and damage while maintaining cellular homeostasis. Catalase deficiency, resulting from various conditions or the use of specific blockers, can lead to several issues within the body. As previously mentioned, the human population faces ongoing exposure to BPA from diverse sources. This literature explores the manifold adverse effects of BPA. Environmental pollutants, including BPA, are identified as significant contributors to female infertility, constituting a substantial portion of infertility cases globally. Therefore, the fundamental objective of this study was to investigate potential adverse outcomes in the female reproductive system resulting from exposure to BPA alone and in the absence of catalase.

Materials and methods

Experimental animal

The study utilized female adult albino mice (Swiss Albino Mice) that were eight weeks old, with a body weight ranging from 20 to 30 grams. Before the initiation of the experiment, the mice underwent a seven-day acclimatization period in the experimental animal house. The animals were housed in cages under standard laboratory conditions, maintaining a favorable temperature of approximately 25±2oC, optimal humidity of 55±5%, and a 12-hour light-dark cycle schedule, with continuous access to water. Throughout the experimental period, the mice had ad libitum access to food and water. To ensure cleanliness and hygiene, daily cleaning routines were implemented, including the removal of feces and spilled food from the cages, aiming to prevent any potential infections. All experimental procedures adhered to ethical guidelines set forth by the Institutional Animal Ethics Committee (IAEC).

Experimental Design

The animals were divided into three groups randomly, each consisting of five rats. Group 1 served as the control, receiving normal saline (10 ml/kg body weight/day, intraperitoneally) throughout the experiment. In Group 2, rats were administered normal saline (10 ml/kg body weight/day, intraperitoneally) for the initial 5 days, followed by treatment with BPA (25 mg/kg body weight/day, intraperitoneally) for the subsequent 9 days, as per Geetharathan and Josthna (2016) (Geetharathan and Josthna, 2016). On the other hand, Group 3 was given the catalase-specific blocker 3-amino-1,2,4-triazole (ATZ; 1 g/kg body weight/day, intraperitoneally) for the first 5 days, following the method outlined by Wood and Legg (1970) (Wood and Legg, 1970). Subsequently, they received BPA (25 mg/kg body weight/twice a day, intraperitoneally) for the next 9 days.

All groups were provided with a control diet comprising 71% carbohydrate, 18% protein, 7% fat, and 4% salt mixture throughout the experimental period (Mukherjee *et al.*, 2006).

Preparation of serum

All groups of animals underwent anesthesia using pentobarbitone sodium (60 mg/kg body weight, administered intraperitoneally) and were subsequently euthanized through cervical dislocation, a recommended physical method. Blood samples were extracted from the heart, and serum was isolated for the assessment of luteinizing hormone (LH), follicle-stimulating hormone (FSH), progesterone and estrogen.

Hormonal assay

Luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were quantified using ELISA kits obtained from Monobind in Lake Forest, CA, USA. Similarly, levels of estrogen and testosterone were assessed utilizing ELISA kits sourced from DRG Inc in Germany.

Results

To analyze whether exposure to BPA had any effect on the reproductive axis, serum gonadotropin levels (FSH and LH) and sex steroid levels (estrogen and testosterone) were measured. Hormonal modulation such as a significant decrease in the serum estrogen level and increase in testosterone levels were observed in the BPA treated group compared to control group. It was evident that pretreatment with a catalase blocker caused a much greater reduction in serum estrogen level and an increment in serum testosterone levels when compared with BPA-treated animals. A remarkable increase in serum LH levels and decrease in FSH level were also observed following BPA exposure, and the alterations were further amplified in BPA-treated animals pretreated with ATZ.

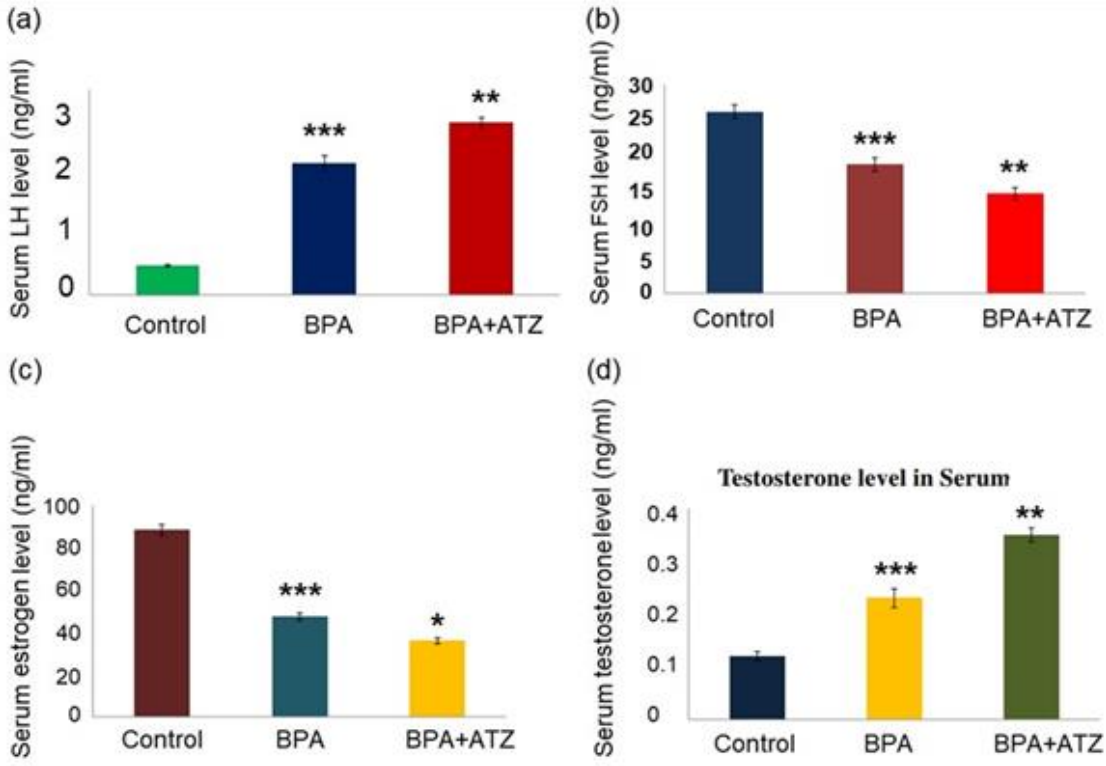

Figure 1: Effect of Bisphenol A (BPA) (1.2mg/kg body weight/day, intraperitoneally) and 3 amino-1,2,4Triazole (ATZ) (2mg/gm body weight/day, intraperitoneally) induced changes on (a) Serum LH level (b) Serum FSH level (c) serum Estrogen level and (d) Serum Testosterone level in female Swiss Albino Mice model. Error bar represents Mean±SE (n=6). Significance based on Kruskal-Wallis test ($P<0.05$). Significance level based on Whitney Multiple U Comparison Test: **$P<0.01$, ***$P<0.001$. BPA wa compared with control group and BPA+ATZ was compared with Control and BPA.

Discussion

An increasing number of studies indicate that exposure to BPA is associated with a variety of disorders of the female reproductive system. The association between BPA exposure and PCOS is supported by both human studies and in vivo animal studies (Tarantino *et al.,* 2013; Kandaraki *et al.*, 2011) The first study conducted by Tarantino et al. (2013) investigated the role of BPA in polycystic ovary syndrome and its association with the liver-spleen axis (Tarantino *et al.,* 2013). Another study by Kandaraki et al. (2011) found elevated serum levels of BPA in women with PCOS, further strengthening the link (Tarantino *et al.,* 2013). Similarly, a study by Fernandez et al. (2010) exposed neonatal rats to BPA, resulting in reproductive and endocrine alterations resembling the polycystic ovarian syndrome in adult rats (Fernández *et al.*, 2010). Indeed, BPA may induce an increase in androgen levels, while androgens decrease BPA clearance. In the present study, we demonstrated the effects of BPA on steroid hormone production in rat ovaries. Additionally, we speculate that catalase possibly plays a significant role in this conjecture. Abnormally high androgen levels may contribute to PCOS, potentially reducing natural negative feedback on GnRH release and LH levels. Fernandez et al. (2010) conducted a study to quantify the change in sex hormone levels in adulthood caused by neonatal exposure to BPA (Fernández *et al.,* 2010). In that study, rats received subcutaneous injections daily of 50 or 500 μg/day from postnatal days 1–10, resulting in higher levels of circulating testosterone and lower levels of estrogen in adulthood (Fernández *et al.*, 2010). Apart from its well-known estrogen-mimetic properties, BPA also seems to have a role in androgen metabolism. There are several lines of evidence indicating the existence of a bidirectional interaction between BPA and androgens (Takeuchi *et al.*, 2006). Specifically, it has been reported that uridine diphosphate-glucuronosyltransferase activity, a liver enzyme involved in BPA clearance from the circulation, is down-regulated by androgens (Takeuchi *et al.*, 2006). Additionally, BPA is a potent SHBG ligand, and, accordingly, in increased concentrations, it displaces androgens from SHBG binding sites, likely leading to increased circulating free androgen concentrations (Pugeat *et al.*, 1996). This

dual impact of BPA on both androgen metabolism and clearance mechanisms further underscores the complexity of its endocrine-disrupting effects and raises important questions about its potential role in hormonal imbalances, such as those observed in conditions like PCOS. Understanding these mechanisms is crucial for unraveling the intricate ways in which BPA disrupts endocrine function and potentially contributes to conditions associated with hormonal imbalances.

Results of this study showed that BPA treatment in vivo caused an increase in testosterone levels. In this study, we demonstrated that testosterone synthesized in ovaries was increased, and estradiol concentrations were suppressed by different concentrations of BPA. This modulation of testosterone and estradiol levels may contribute to decreased circulating LH levels. The observed changes in hormone levels suggest a potential mechanism by which BPA disrupts the endocrine system, affecting key components of androgen and steroid hormone production in the context of reproductive function. Understanding these effects is crucial for comprehending the broader implications of BPA exposure on reproductive health.

An earlier molecular docking study revealed that BPA interacts with catalase and promotes cytotoxicity in different cell types (Zhang *et al.,* 2016). Another earlier study found that among antioxidant enzymes, catalase was maximally inhibited by BPA (Jayakanthan *et al.,* 2015). These findings suggest a potential mechanism by which BPA may induce oxidative stress by interacting with and inhibiting catalase, a crucial antioxidant enzyme. The inhibition of catalase could lead to an imbalance in the cellular redox state, contributing to oxidative damage and cytotoxicity. Understanding the molecular interactions between BPA and key enzymes like catalase provides valuable insights into the potential mechanisms underlying BPA-induced cellular toxicity and oxidative stress. In this study, BPA treatment caused more ovarian androgen production in ATZ pretreated female mice. Earlier observations indicate that theca-interstitial cells are sensitive to the level of reactive oxygen species (ROS), and the reduction of oxidants triggers apoptosis. These findings may have translational-clinical relevance to polycystic ovary syndrome (PCOS), a condition associated with excessive growth and activity of theca-interstitial cells. It is tempting to speculate that antioxidants may have potential therapeutic value, and one possible mechanism of their action may be related to the reduction of oxidative stress and apoptosis. This concept is supported by clinical studies demonstrating beneficial effects of one antioxidant, N-acetylcysteine, on various aspects of PCOS, including the restoration of gonadal function.

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