The Oxidant Effect of Bisphenol A (BPA) Can be Decoupled from its Endocrine Disruptor Property

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Abstract. Bisphenol A (BPA) is an environmental defilement released mainly from polycarbonate plastic and epoxy resins. The main toxicological impact of BPA is its endocrine disruptor activities. Its structural features confer the ability to bind to both estrogen receptor (ER) subtypes. Furthermore, we recently reported that BPA aggravates male reproductive hormones. In addition to its endocrine disruptor properties, we have also reported that BPA possesses oxidant activity which is able to trigger oxidative stress. Several types of research previously reported that oxidative stress may cause hormonal imbalance and vice versa. However, the relation of both the toxicological properties of BPA is poorly understood. In this study, we found that oral testosterone undecanoate treatment in BPA-induced rats does not prevent decreasing serum superoxide dismutase, glutathione peroxidase, and catalase, and increasing serum malondialdehyde. Oral N-acetyl cysteine (NAC) in BPA-induced rats also does not attenuate decreasing total testosterone levels. These results suggest that the oxidant effect and endocrine disruptor property of BPA can be separated and might not interfere with one another. Therefore, future treatment in any pathological condition resulting from BPA exposure has to be carried out with more comprehensive approaches rather than focusing on its endocrine disruptor activity.

1. Introduction

Bisphenol A (BPA, 2, 2-bis (4-hydroxyphenyl) propane; CAS RN 80-05-7) is an environmental defilement released mainly from polycarbonate plastic and epoxy resins [1]. In the chemical industry, BPA is used as a basic material that is used as a component of many consumer products and can be released then accumulate in the body [2]. The main toxicological impact of BPA is its endocrine disruptor activities. Its structural features confer the ability to bind to both estrogen receptor (ER) subtypes, hence possesses either agonist or antagonist effect via ER-dependent signaling pathways [3]. Previously, lots of studies have reported that BPA exposure deteriorates reproductive function and causes abortion [4].
Recently, a study found that BPA aggravates male reproductive hormones, by causing disruption of the hypothalamic-pituitary-testicular axis and leads to reduces sperm quality [5]. Similar to its activity to mimic estrogen, BPA can also bind to the androgen receptor (AR) and act as its antagonist via competitive inhibition of the action of endogenous androgen [6]. The study has also shown that BPA can bind thyroid hormone receptors [7]. The consequene of the androgen-like activity of BPA, it reduces the levels endogenous testosterone by directly affects not only the Leydig cells (inhibits expressions of steroidogenic enzymes and cholesterol carrier protein) but also the pituitary gland (prevent LH secretion) [8].

In addition to its endocrine disruptor properties, BPA possesses oxidant activity which is able to trigger oxidative stress. A growing body of evidence indicates that BPA induces reactive oxygen species (ROS) production through the enzymatic (such as peroxidase and CYP450) and non-enzymatic (peroxynitrite and OCI/HOCl) formation of phenoxy radicals (Reviewed in [9]). Because the enzymatic free radical scavengers, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GPx), play critical roles to ameliorate oxidative damage, BPA can cause depletion in these systems. Indeed, the study reported that BPA depletes GSH [10], and reduces SOD, CAT and GPx activity [11]. Another study reported that BPA increased nitric oxide (NO) levels but not reactive oxygen species (ROS), therefore causing the nitrosylation of Keap1 and act as electrophile [12]. The reduction of enzymatic antioxidant activities has a strong relationship with the induction of ROS by BPA [9].

Several types of research previously reported that oxidative stress may cause hormonal imbalance and vice versa [13–15]. Hitherto, however, the relation of both the toxicological properties of BPA is poorly understood. In this study, we examined the relation and dependency of the BPA effect as an endocrine disruptor and its activity as oxidant.

2. Materials and Methods

2.1 Animals
Forty twelve-week-old experimentally naive male Wistar albino rats with an average initial body weight of 180 ± 14 g were used. Animals were housed in Animal House of Faculty of Veterinary Medicine, Udayana University, Denpasar, Indonesia under environmentally controlled conditions (12 hr light/dark cycle; 23 ± 2 °C). Food and water were given ad libitum throughout the experiment. Animals were allowed to adjust to laboratory conditions for seven days. The protocols used were conformed to Ethical Guidelines for the Use of Animals in Research and were approved by the committee on the ethics of animal experiments in the Faculty of Veterinary Medicine, Udayana University.

2.2 Experimental design
After seven days of adaptation, all rats were randomly and equally divided into four groups, containing ten rats in each group as follows: negative control (P0) group that was treated with oral distilled water as a placebo, BPA-treated (P1) group that was treated with oral BPA (Sigma Chemical Co, St. Louis, MO, USA) at 400 mg/kg.BW/day for 21 days, the BPA- and testosterone-treated (P2) group that was treated with oral BPA at 400 mg/kg.BW/day and oral testosterone undecanoate (Schering-Plough, Kenilworth, NJ, USA) at 4 mg/kg.BW/day for 21 days, and the BPA- and NAC-treated (P3) group that was treated with oral BPA at 400 mg/kg.BW/day and oral N-acetyl cysteine (Sigma Chemical Co, St. Louis, MO, USA) at 600 mg/kg.BW/day for 21 days. All chemical was administered by intragastric tube.

2.3 Sample Collection
Before the treatment and at the end of the study, animals were anesthetized with intraperitoneal injection of a mixture of ketamine and xylazine at 100 mg/kg and 10 mg/kg respectively; peripheral blood was collected from the retro-orbital sinus according to the previously described method [16]. Blood was sampled at 2 hours after the last treatment of every group. After allowing blood to clot on
ice, the serum sample was separated by centrifugation at 3000 × g for 15 min. The serum was collected and stored at -20°C for further examination.

2.4 Biochemical Analysis

All biochemical analysis was performed using the enzyme-linked immunosorbent assay (ELISA) methods with the commercially available kit, except for the MDA analysis. Serum total testosterone levels were determined by Rat Testosterone ELISA kit (Bioassay Technology Laboratory, Catalog No. E0259Ra). Serum superoxide dismutase, glutathione peroxidase, and catalase were determined by Rat SOD ELISA kit (Bioassay Technology Laboratory, Catalog No. E1444Ra), were determined by Rat GPx ELISA kit (Bioassay Technology Laboratory, Catalog No. E1172Ra), and were determined by Rat CAT ELISA kit (Bioassay Technology Laboratory, Catalog No. E0869Ra) respectively. The stepwise of any biochemical examination were according to the manufacturer’s instructions.

MDA level was examined by thiobarbituric acid reactive substances assay (TBRAS) according to the previously described method [17]. In brief, a working solution containing 15% trichloroacetic acid, 0.375% thiobarbituric acid, and 0.25 N hydrochloric acid was prepared. For each sample, 250 μL serum and 500 μL working solution were mixed and placed in boiling water for 10 min. After the samples cooled, 25 μL of 5 mol/L HCl was added (final pH 1.6-1.7), and the reaction mixture was extracted by agitation for 5 min with 3.5 mL of n-butanol, and butanol phase was separated by centrifugation at 1500 × g for 10 min. The precipitate was discarded, and the suspension used to measure the absorbance at 525 nm for excitation and 547 nm for emission. Protein concentration was measured using BSA as a standard by the method according to Lowry et al. (1951) [18].

2.5 Statistical analysis

Data are presented as mean ± SD. Statistical differences were determined by either paired t-test or one-way ANOVA with Turkey post-hoc test. p < 0.05 was considered statistically significant.

3. Results

The deleterious effect of BPA on male reproductive function has been extensively reported. In this study, we confirmed that the 400 mg/kg.BW/day of oral BPA treatment for 21 days modestly decreases the free testosterone levels. Additionally, while no changes were observed in the enzymatic antioxidant levels, the BPA-treated group experienced depletion in all enzymatic antioxidants tested confirming the oxidant effect of BPA. Indeed, the levels of MDA, which is the marker of oxidative lipid peroxidation, was also raised significantly (table 1).

| Table 1. The effect of BPA on testosterone, enzymatic antioxidant, and oxidative damage marker. |
|---------------------------------------------------------------|
| **P0 Group** | **Post-test** | **Pre-test** | **Post-test** |
|---------------------------------|-----------------|-----------------|
| Total Testosterone (nmol/ml) | 0.312 ± 0.02    | 0.309 ± 0.01    | 0.318 ± 0.02    | 0.275 ± 0.01* |
| Serum SOD (ng/ml) | 8.906 ± 0.78    | 8.576 ± 0.88    | 8.717 ± 0.79    | 7.573 ± 0.26* |
| Serum GPx (nmol/ml) | 26.532 ± 1.45   | 27.218 ± 2.82   | 25.452 ± 2.66   | 18.908 ± 2.62** |
| Serum CAT (µmol/ml) | 1.835 ± 0.12    | 1.816 ± 0.02    | 1.821 ± 0.24    | 1.129 ± 0.18** |
| Serum MDA (µmol/ml) | 2.671 ± 0.33    | 2.626 ± 0.16    | 2.753 ± 0.27    | 3.281 ± 0.13* |

Data were given as mean ± SD (*p < 0.05; **p < 0.01 significantly different from the pre-test counterparts)

Given the fact that BPA possesses both endocrine disruptor and oxidant effect, we intrigued to examine the dependency and relation of both properties of BPA. It is due to several studies that reported the oxidative stress may cause hormonal imbalance and vice versa [13–15]. Surprisingly, oral testosterone undecanoate treatment in BPA-induced rats does not prevent decreasing serum superoxide
dismutase, glutathione peroxidase, and catalase, and increasing serum malondialdehyde (figure 1). Next, we used N-acetyl cysteine (NAC), a well-established antioxidant [19], to ameliorates the oxidant effect of BPA. Concomitantly, the treatment of oral NAC in BPA-induced rats also does not attenuate decreasing total testosterone levels (figure 2). This study, therefore, provides evidence, at least in part, that the oxidant effect and endocrine disruptor property of BPA can be decoupled.

**Figure 1.** The effect of oral testosterone undecanoate on enzymatic antioxidant and oxidative damage marker of BPA-induced rats. (A) The level of SOD, (B) GPx level, and (C) The amount of CAT was examined from the serum sample and measured with ELISA methods. (D) The level of MDA was examined by the TBRAS assay. The values are expressed as a percentage to their pre-test counterparts. All graphs are expressed as the mean ± S.D (n = 10). *p<0.05, **p<0.01, N.S.= not significant versus indicated group.
4. Discussion
The BPA has the endocrine disruptive activity both in male and female organism. This and other studies have extensively and supportively found that BPA abrogates the endocrine homeostasis, especially the reproductive-related anabolic hormones. BPA and its derivates are considered as xenoestrogens due to structural and biological similarities to the endogenous estrogen, regardless of its 1000- to 2000-fold weaker affinity to the ERs than 17β-estradiol (E2) [20].

In addition to its xenoestrogen activity, BPA is also capable of binding to other nuclear receptors, such as the androgen receptor (AR). BPA binding to AR provides the antagonist role via competitive inhibition of the action of endogenous androgen [6]. The consequence of the androgen-like activity of BPA, it reduces the levels of endogenous testosterone by directly affects the Leydig cells, causing inhibition on expressions of steroidogenic enzymes and cholesterol carrier protein. BPA also directly inhibits the pituitary gland to produce and secrete LH [8]. Indeed, we found lower testosterone levels in the group treated with BPA. Similarly, multiple studies show that exposure to low-dose BPA was related to decreased testosterone levels in rats [21], mice [22], and humans [23].

In order to study the oxidant effect of BPA and the possible relation to endocrine disruption property, we determined the level of antioxidant enzymes and the MDA concentrations due to its visibility and widely proven markers oxidative stress [24]. In agreement with abundant proves in the oxidant effect of BPA, we found that BPA results in lower antioxidant enzymes and higher MDA levels. It is due to BPA exposure that triggers the formation of intracellular peroxides and mitochondrial superoxide in time- and dose-dependent manner [25]. The generation of free radicals by BPA has been reported in a large number of studies and with doses ranging from $10^{-12}$ to $10^{-4}$ M (Reviewed in [9]). Due to the role of the enzymatic antioxidant as radical scavengers, BPA can cause depletion in these systems. Supportedly, the study reported that BPA decreases GSH [10], and reduces SOD, CAT and GPx activity [11]. Accumulatively, these data strongly support the prooxidant role of BPA.

Despite well documented effect of endocrine disruption and oxidant activity of BPA separately, the relation of these two activities is poorly defined. Here, we found that oral testosterone undecanoate treatment in BPA-induced rats does not prevent decreasing serum superoxide dismutase, glutathione peroxidase, and catalase, and increasing serum malondialdehyde. The amelioration of the endocrine

![Figure 2](image_url)
disruption effect of BPA does not interfere with its oxidant activity. The same goes for oral N-acetyl cysteine (NAC) that does not attenuate decreasing total testosterone levels. These results suggest that the oxidant effect and endocrine disruptor property of BPA can be separated and might not interfere with one another. To the best of our knowledge, we are the first to report the independent attribute of distinct BPA activity.

5. Conclusion
Taken together, our results suggesting that the oxidant effect and endocrine disruptor property of BPA can be decoupled and affect the homeostasis independent to one another. Hence, future treatment in any pathological condition resulting from BPA exposure has to be carried out with more comprehensive approaches rather than focusing on either oxidant alone or its endocrine disruptor activity.

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