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Mitochondrial signaling pathway is also involved in bisphenol A induced germ cell apoptosis in testes

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ABSTRACT

Bisphenol A (BPA) is a potential endocrine disruptor and testicular toxicant. An earlier study showed that BPA-induced germ cell apoptosis through the Fas/FasL apoptotic pathway. In the present study, we aimed to investigate whether the mitochondrial pathway is also involved in the process of BPA-mediated germ cell apoptosis in testes. Male mice were administered with BPA (160 or 480 mg/kg) by gavage daily from postnatal day 35 (PND35) to PND49. Germ cell apoptosis in testes was determined by terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling (TUNEL). As expected, the number of TUNEL+ germ cells per tubule and the percentage of tubules with TUNEL+ germ cells were significantly increased in testes of mice treated with BPA during puberty. TUNEL+ germ cells were observed mainly in stages VII–VIII seminiferous tubules in testes. An increase in the level of Fas and FasL was observed in testes of mice exposed to BPA during puberty. In addition, pubertal BPA exposure evoked the activation of caspase-8 and caspase-3 in testes. Interestingly, pubertal BPA exposure also caused the translocation of cytochrome c from mitochondria into cytosol. In addition, pubertal BPA exposure upregulated the level of Bax and active caspase-9 in testes. Taken together, these results suggest that pubertal BPA exposure induces germ cell apoptosis in testes through not only the Fas/FasL signaling pathway but also the mitochondrial apoptotic pathway.

1. Introduction

In the seminiferous tubules, apoptosis is a normal feature of spermatogenic lineages and testicular homeostasis. During various stages of mammalian spermatogenesis, germ cell apoptosis occurs to remove abnormal spermatogenic cells (Print and Loveland, 2000; Rodriguez et al., 1997; Russell et al., 2002). On the other hand, some harmful factors, such as hormone deprivation, heat and radiation exposure, aggravate germ cell apoptosis in testes (Vera et al., 2006; Hikim et al., 2003; Embree-Ku et al., 2002). In addition, some of endocrine disruptors have been demonstrated to induce germ cell apoptosis in testes. According to a recent report, mono-(2-ethylhexyl) phthalate, a well-known endocrine disruptor, reduced the number of germ cells by increasing apoptosis without change in testosterone synthesis in human fetal testes (Lambrot et al., 2009). Additional study showed that diethylstilbestrol, a synthetic estrogen, induced spermatogenic cell apoptosis in male hamster (Ma et al., 2008). Several studies have demonstrated that excess germ cell apoptosis may disrupt normal testicular development and spermatogenesis (Francavilla et al., 2000; Sawhney et al., 2005).

The signaling events leading to apoptosis can be divided into two major pathways, either Fas and Fas ligand (FasL) or mitochondrial pathway. Fas/FasL system has been shown to be the major inducer of germ cell apoptosis. Several studies have demonstrated that Fas and FasL play an important in the apoptotic death of germ cells that results from reduced intratesticular testosterone levels or hormone deprivation (Nandi et al., 1999; Pareek et al., 2007). On the other hand, mitochondrial signaling pathway is also involved in germ cell apoptosis in testes. Translocation of cytochrome c from mitochondria into cytosol is the primary event in mitochondrial signaling pathway for apoptosis (Kluck et al., 1997). According to several earlier studies, the mitochondria-dependent pathway is the key apoptotic pathway for heat-induced male germ cell death in mice (Hikim et al., 2003; Vera et al., 2004).

Bisphenol A (BPA) is one of the highest volume chemicals produced worldwide, with over 6 billion pounds produced each year and over 100 tons released into the atmosphere by yearly production (Vandenberg et al., 2009). Importantly, BPA can be released from polycarbonate drinking bottles including polycarbonate baby bottles and reusable water bottles (Vandenberg et al., 2007; Le et al., 2008). Numerous studies demonstrated that BPA is a poten-

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tial endocrine disruptor (Welshons et al., 2006). Although BPA is a weak environmental estrogen for its relatively low affinity for the nuclear estrogen receptors, results from recent studies have revealed a variety of pathways, such as membrane steroid receptors, through which BPA can stimulate cellular responses at a very low concentration (Alonso-Magdalena et al., 2005). On the other hand, BPA is also a testicular toxicant. According to an earlier study, the absolute weights of the testes and seminal vesicles and epididymal sperm counts were significantly reduced in adult mice intragastrically administered with 25 μg/kg of BPA daily for 28 days (Al-Hiyasat et al., 2002). Neonatal BPA exposure caused a reversible damage on testicular development and spermatogenesis in rats and mice (Toyama and Yuasa, 2004). A recent study showed that BPA-induced germ cell apoptosis through the Fas/FasL apoptotic pathway (Li et al., 2009). However, whether mitochondrial signaling pathway is also involved in BPA-mediated germ cell apoptosis in testes remains unclear.

In the present study, we aimed to investigate whether the mitochondrial pathway is involved in the process of BPA-mediated germ cell apoptosis in testes. We found that pubertal exposure to a high dose of BPA-induced germ cell apoptosis in testes. BPA-mediated germ cell apoptosis in testes depends on not only the Fas/FasL signaling but also the mitochondrial pathway.

2. Materials and methods

2.1. Chemicals

Bisphenol A (BPA) was purchased from Sigma Chemical Co. (St. Louis, MO). All other reagents were from Sigma or as indicated in the specified methods.

2.2. Animals and treatments

Male CD-1 mice (4-week-old, 18–22 g) were purchased from Beijing Vital River whose foundation colonies were all introduced from Charles River Laboratories, Inc. All animals were allowed free access to food and water at all times and were maintained on a 12-h light/dark cycle in a controlled temperature (20–25 °C) and humidity (50 ± 5%) environment. Mice were administered with different doses of BPA (160 or 480 mg/kg) by gavage daily from PND35 to PND49. The maximum nontoxic dose and minimum toxic dose of BPA are estimated about 200 mg/kg/day in rats and mice (Takahashi and Oishi, 2003). Moreover, the recent study showed no significant reproductive effect was observed in mice were exposed to BPA at 160 mg/kg (Li et al., 2009). Accordingly, the doses of BPA used in present study were determined. The corn oil treated male mice served as controls. All mice were fed a standard chow diet. At PND50, the testes were excised, dissected, and weighted. The means of the absolute weight of testes and the relative weight of testes (mg/g BW) was calculated. The testes then divided in two parts: left one was kept at −80 °C for subsequent immunoblotting. The other part of the testes was immersed in modified Davidson's fluid (mDF) (Latendresse et al., 2002) for 12–24 h for testicular histology and apoptosis analysis. All procedures on animals followed the guidelines for humane treatment set by the Association of Laboratory Animal Sciences and the Center for Laboratory Animal Sciences at Anhui Medical University.

2.3. Terminal dUTP nick-end labeling (TUNEL) staining

For the detection of apoptosis, paraffin-embedded sections were stained with the TUNEL technique using an in situ apoptosis detection kit (Promega) according to the manufacturer's protocols. To assess apoptosis in testicular cells, 200 seminiferous tubules were observed in each section at a magnification of 400×. Seminiferous tubules were chosen according to the same criterion. A histogram of the number of TUNEL-positive germ cells per seminiferous tubule and the percentages of the number of seminiferous tubules containing TUNEL-positive germ cells were analyzed.

2.4. Immunoblotting

For immunoblotting, testicular samples (100 mg) were homogenized in lysis buffer containing 50 mM Tris–HCl (pH 7.4), 150 mM NaCl, 1 mM EDTA, 1% Triton X-100, 1% sodium deoxycholate, 0.1% sodium dodecylsulfate (SDS), and 1 mM phenylmethylsulfonyl fluoride. Samples were then centrifuged at 15,000 × *g* for 15 min. Supernatants were collected and the protein concentration was measured by BCA protein assay kit (Pierce, Rockford, IL). Protein extracts from each sample were added to a gel loading buffer (100 mM Tris, pH 6.8, 20% glycerol, 200 mM DTT, 4% SDS, 0.03% bromophenol blue) and boiled for 5 min. Proteins (50 μg/sample) in loading buffer were subjected to electrophoresis in 10% SDS-polyacrylamide gel for 3 h. The gel was transferred electrophoretically onto a polyvinylidene fluoride membrane (Immobilon-P; Millipore Corp., Bedford, MA, USA) and blocked in 5% nonfat powdered milk in Dulbecco's PBS (DPBS) overnight at 4 °C. The membranes were then incubated for 2 h with rabbit polyclonal antibody against Fas (sc-1024, Santa Cruz Biotechnology, USA) or FasL (sc-834, Santa Cruz Biotechnology, USA) or Bcl-2 (sc-492, Santa Cruz Biotechnology, USA) or Bax (sc-526, Santa Cruz Biotechnology, USA) or β-actin (bs-0061R, Beijing Biosynthesis Biotechnology, Beijing, China) at room temperature. After washes in DPBS containing 0.05% Tween-20 four times for 10 min each, the membranes were incubated with goat anti-rabbit IgG antibody for 2 h. The membranes were then washed for four times in DPBS containing 0.05% Tween-20 for 10 min each, followed by signal development using an enhanced chemiluminescence (ECL) detection kit from Pierce (Pierce Biotechnology, Rockford, IL).

2.5. Abundance of mitochondrial and cytosolic cytochrome c and Bax

Testicular tissues were washed with 10 ml of ice-cold PBS buffer and centrifugated at 600 × *g* for 5 min at 4 °C. The supernatants were removed, and the pellets were resuspended on ice with 1 ml of Cytosol Extraction Buffer Mix (BioVision, Mountain View, CA, USA) containing DTT and protease inhibitors. Ten minutes later, these tissues were homogenized by an ice-cold glass Dounce tissue grinder on ice for 15 times, and were then centrifugated at 700 × *g* for 10 min at 4 °C to remove the pellets. The supernatants were further centrifugated at 100,000 × *g* for 30 min at 4 °C to separate the cytosolic and the mitochondrial fractions of the cytoplasmic proteins. The mitochondrial fraction were finally resuspended with Mitochondrial Extraction Buffer Mix (BioVision, Mountain View, CA, USA) containing DTT and protease inhibitors, and vortexed for 10 s to obtain the mitochondrial protein. To determine the relative abundance of cytochrome c and Bax in the compartments of mitochondria and cytosol, 50 μg of proteins from cytosolic and mitochondrial extracts were resolved by 15% SDS-PAGE and underwent immunoblotting analysis as described above.

2.6. Testicular histology

Two cross sections from each testis were embedded in parafilm using standard procedures performed by Pathological Lab at Anhui Medical University. Paraffin-embedded tissues were serially sectioned. At least two nonserial sections were stained with hematoxylin and eosin (H&E) using standard procedures for morphological analyses.

2.7. Statistical analysis

For immunoblotting, developed films were scanned and band intensities were analyzed using the public domain NIH Scion Image Program. Fas, FasL, Bcl-2 and Bax were normalized to β-actin level in the same samples. The densitometry unite of the control was assigned as 1. All quantified data were expressed as means ± SD at each point. $P < 0.05$ was considered statistically significant. ANOVA and the Student–Newmann–Keuls post hoc test were used to determine differences among different groups.

3. Results

3.1. Effects of pubertal BPA exposure on testis weights

As expected, the mean body weight was significantly decreased in mice exposed to BPA (Table 1). The effects of pubertal BPA

Table 1
Body weight, absolute testis weight and relative testis weight.

Dose (mg/kg/day)	Body weight (g) (means ± SD, $n = 12$)	Absolute testis weight (g) (means ± SD, $n = 12$)	Relative testis weight (mg/g BW) (means ± SD, $n = 12$)
0	31.0 ± 1.23	0.25 ± 0.02	8.14 ± 0.71
160	26.0 ± 2.62**	0.22 ± 0.02*	8.49 ± 0.66
480	26.3 ± 5.33*	0.21 ± 0.02*	8.38 ± 1.47

* $P < 0.05$ as compared with control.
** $P < 0.01$ as compared with control.

Table 2
Distribution of TUNEL+ seminiferous tubules in different stages.

Dose (mg/kg/day)	The number of TUNEL+ seminiferous tubules in different stages (means ± SD, $n = 12$)		
	I–VI	VII–VIII	IX–XII
0	3.17 ± 1.33	8.17 ± 2.48	1.83 ± 1.72
160	4.50 ± 3.27	16.67 ± 9.99*	3.67 ± 3.88
480	5.20 ± 3.90	19.80 ± 10.26*	2.00 ± 2.12

* $P < 0.05$ as compared with control.

exposure on testis weights were analyzed. Results showed that the absolute weight of testes was significantly decreased in mice exposed to BPA during puberty (Table 1). However, pubertal BPA exposure had little effect on the relative weight of testes (Table 1).

3.2. Effects of pubertal BPA exposure on testicular histology

The effects of pubertal BPA exposure on testicular histology are shown in Fig. 1. The morphology of testes in control mice was normal (Fig. 1A). Pubertal BPA exposure slightly reduced the layers of spermatogenic cells and disturbed the array of spermatogenic cells (Fig. 1C, E and F). No large vacuoles or complete spermatogenic failure were observed in testes of mice exposed to BPA during puberty.

3.3. Effects of pubertal BPA exposure on germ cell apoptosis in testes

The effects of pubertal BPA exposure on germ cell apoptosis in mouse testes are presented in Fig. 2. As shown in Fig. 2D, the number of TUNEL+ germ cells per tubule was significantly increased in testes of mice exposed to a high dose of BPA. In addition, the percentage of tubules with TUNEL+ germ cells were significantly increased in mice exposed to a high dose of BPA as compared with controls (Fig. 2E). The distribution of seminiferous tubules with TUNEL+ germ cells was analyzed. As shown in Table 2, TUNEL+ germ cells were observed mainly in stages VII–VIII seminiferous tubules. The effects of pubertal BPA exposure on the level of procaspase-3 and active caspase-3 in testes were presented in Fig. 3. As shown in Fig. 3A, the expression of procaspase-3 in testes was significantly upregulated in mice exposed to a high dose of BPA. In addition, pubertal BPA exposure significantly increased the level of active caspase-3 in testes (Fig. 3B).

3.4. Effects of pubertal BPA exposure on Fas/FasL apoptotic pathway

The effects of pubertal BPA exposure on Fas/FasL apoptotic pathway in testes were analyzed. As shown in Fig. 4, pubertal BPA exposure not only significantly upregulated the expression of FasL in testes, the level of testicular Fas was significantly increased in testes of mice exposed to BPA in a dose-dependent manner. The effects of pubertal BPA exposure on caspase-8 in testes are presented in Fig. 5. As expected, the level of active caspase-8 in testes was significantly increased in mice exposed to different doses of BPA during puberty.

3.5. Effects of pubertal BPA exposure on mitochondrial apoptotic pathway

The effects of pubertal BPA exposure on mitochondrial pathway were analyzed. Results showed that the level of Bax in testes was significantly increased in mice exposed to BPA in a dose-dependent manner (Fig. 6A). As shown in Fig. 6B, the expression of Bcl-2 was slightly upregulated in testes of mice exposed to a high dose of BPA (480 mg/kg), whereas no significant difference on the level of Bcl-2 in testes was observed between control mice and mice exposed to a low dose of BPA (160 mg/kg) during puberty. The level of Bax in mitochondrial and cytosolic fractions of testicular lysates was analyzed. Results showed that the level of Bax in mitochondrial fractions of testicular lysates was significantly increased in mice exposed to a high dose of BPA (480 mg/kg)

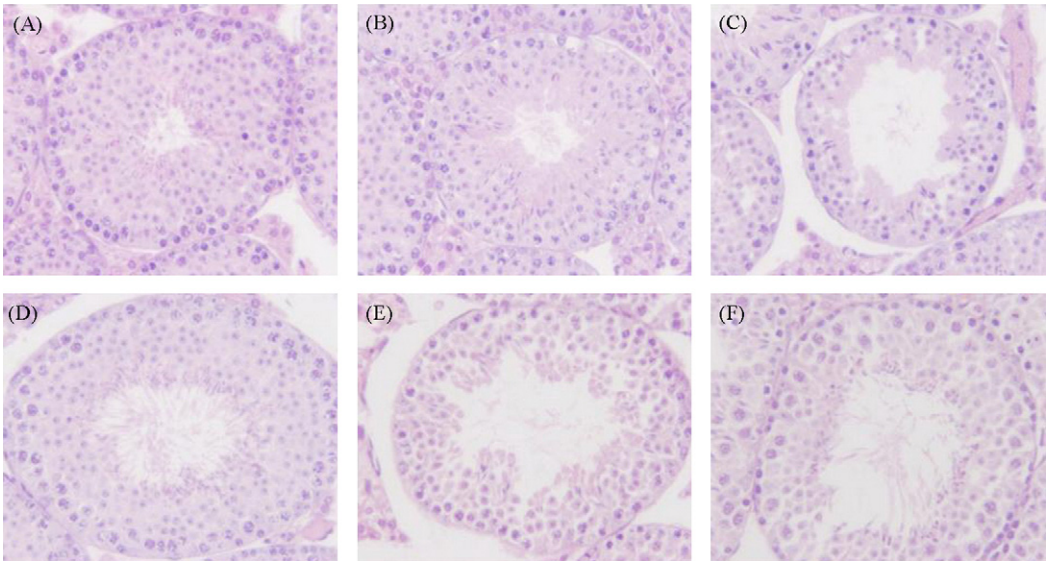

Fig. 1. Effects of pubertal BPA exposure on testicular histology. Testis sections were stained with H&E. (A–C) Seminiferous tubules in stages I–VI from testes of (A) control, (B) the low dose of BPA (160 mg/kg) and (C) the high dose of BPA (480 mg/kg) treated mice. (D–F) Seminiferous tubules in stages VII–VIII from testes of (D) control, (E) the low dose of BPA (160 mg/kg) and (F) the high dose of BPA (480 mg/kg) treated mice.

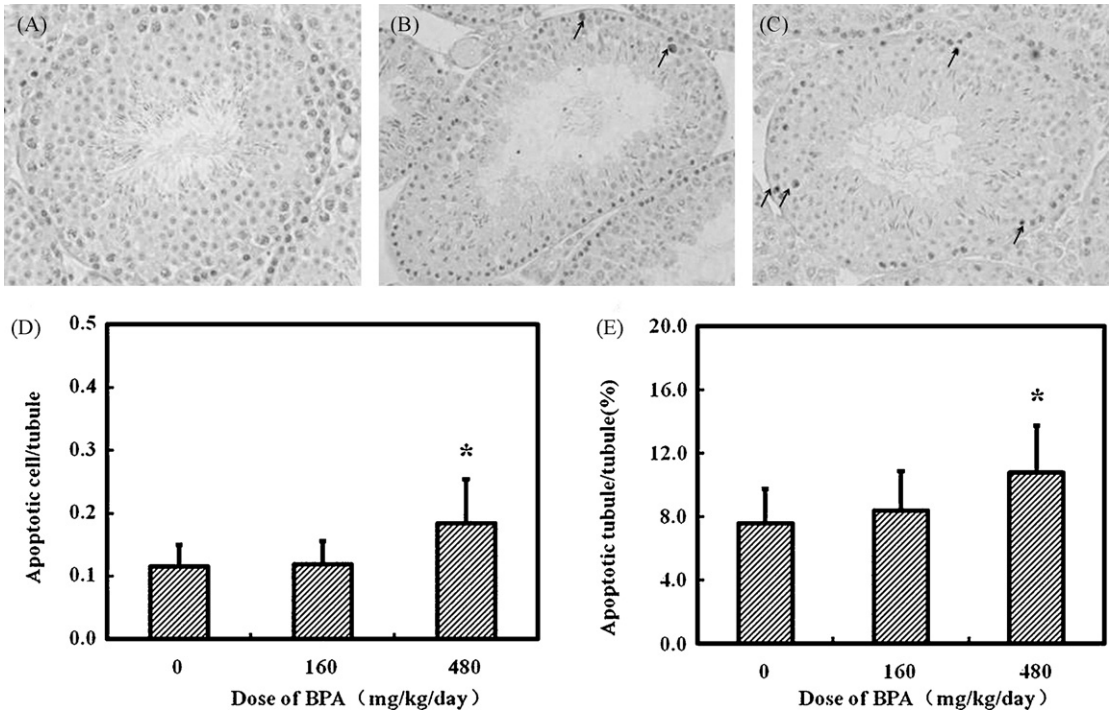

Fig. 2. Effects of pubertal BPA exposure on germ cell apoptosis in testes. Germ cell apoptosis was detected by TUNEL staining. Seminiferous tubules in stages VII–VIII from testes of (A) control, (B) the low dose of BPA (160 mg/kg) and (C) the high dose of BPA (480 mg/kg) treated mice. Arrows showed TUNEL-positive cells. (D) The number of TUNEL+ germ cells per seminiferous tubule. (E) The percentages of seminiferous tubule with TUNEL+ germ cells. Data were expressed as means ± SD of twelve samples from twelve male mice. $^{*}P < 0.05$ as compared with controls.

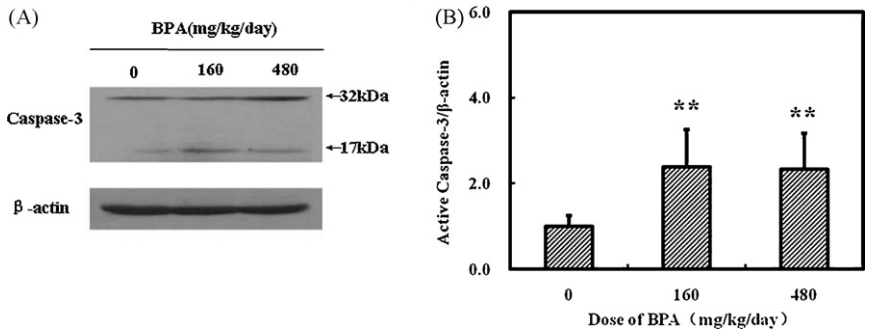

Fig. 3. Effects of pubertal BPA exposure on caspase-3 in testes. (A) The levels of procaspase-3 (32 kDa) and active caspase-3 (17 kDa) were measured in testes using immunoblotting. Quantitative analysis of scanning densitometry for active caspase-3 (B) was performed. All data were expressed as means ± SD of six samples from six different mice. $^{**}P < 0.01$ as compared with controls.

(Fig. 7B), whereas no significant difference on the level of Bax in cytosolic fractions was observed between BPA-treated mice and controls (Fig. 7A). The release of cytochrome c from mitochondria into cytosol and subsequent activation of caspase-9 is a key step in the mitochondrion-dependent apoptotic pathway. The effects of pubertal BPA exposure on translocation of cytochrome c from mitochondria into cytosol are presented in Fig. 7. As expected, cytosolic cytochrome c was significantly increased in testes of mice treated with BPA during puberty (Fig. 7A). By contrast, the level of mitochondrial cytochrome c was significantly decreased in testes of mice exposed to BPA (Fig. 7B). The effects of pubertal BPA exposure on the activation of caspase-9 are presented in Fig. 8. Results showed that the level of active caspase-9 was significantly increased in testes of mice exposed to BPA.

4. Discussion

In the present study, we found that the absolute testis weight were significantly decreased in mice exposed to BPA during puberty. However, pubertal BPA exposure had little effect on the relative weight of testes. Thus, it is likely that the decreased testis weight might be secondary to BPA-induced systemic toxicity. Indeed, no large vacuoles or complete spermatogenic failure were observed in testes of mice treated with BPA although pubertal BPA exposure slightly reduced the layers of spermatogenic cells and disturbed the array of spermatogenic cells. These results were in agreement with the results from other laboratory, in which no significant impairments on testicular histopathology were observed in mice orally administered with as high as 600 mg/kg/day of BPA (Tyl et al., 2008).

Germ cell apoptosis is a more sensitive marker for testicular histopathology. In the present study, we investigated the effects of pubertal BPA exposure on germ cell apoptosis in testes using TUNEL assay. We found that the number of TUNEL-positive germ cells per tubule was significantly increased in mice exposed to BPA during puberty. In addition, pubertal BPA exposure significantly increased the percentage of tubules with TUNEL-positive germ cells in testes. Interestingly, the present results show that

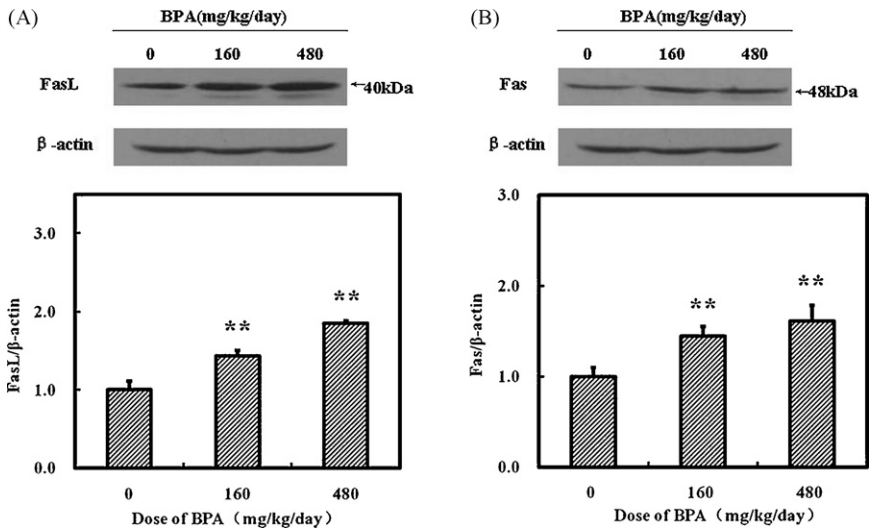

Fig. 4. Effects of pubertal BPA exposure on the expression of Fas and FasL in testes. The protein expression of Fas and FasL in testes was measured using immunoblotting. (A) FasL; (B) Fas. All data were expressed as means ± SD of six samples from six different mice. $^{**}P < 0.01$ as compared with controls.

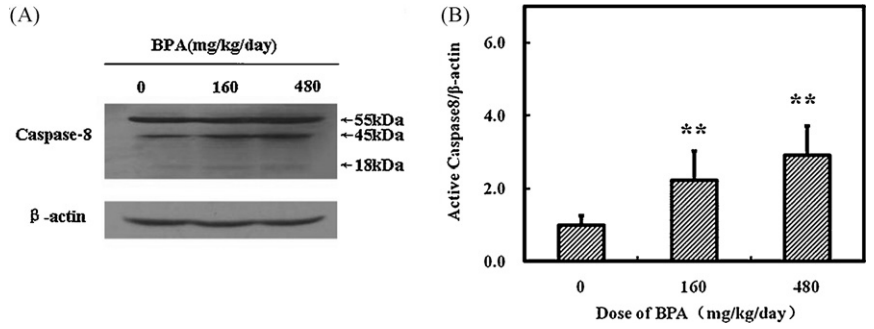

Fig. 5. Effects of pubertal BPA exposure on the level of caspase-8 in testes. (A) The level of active caspase-8 (18 kDa) was measured in testes using immunoblotting. (B) All data were expressed as means ± SD of six samples from six different mice. $^{**}P < 0.01$ as compared with controls.

TUNEL-positive germ cells were observed mainly in stages VII–VIII seminiferous tubules in testes. An earlier study demonstrated that reduced levels of intratesticular testosterone resulted in a cell and stage specific apoptosis of round spermatids and spermatocytes in stages VII and VIII tubules (Bartlett et al., 1986). Indeed, BPA is a potential endocrine disruptor. According to an earlier report, serum level of testosterone was significantly decreased in rats exposed to BPA from PND21 to PND35 (Akingbemi et al., 2004). A recent study

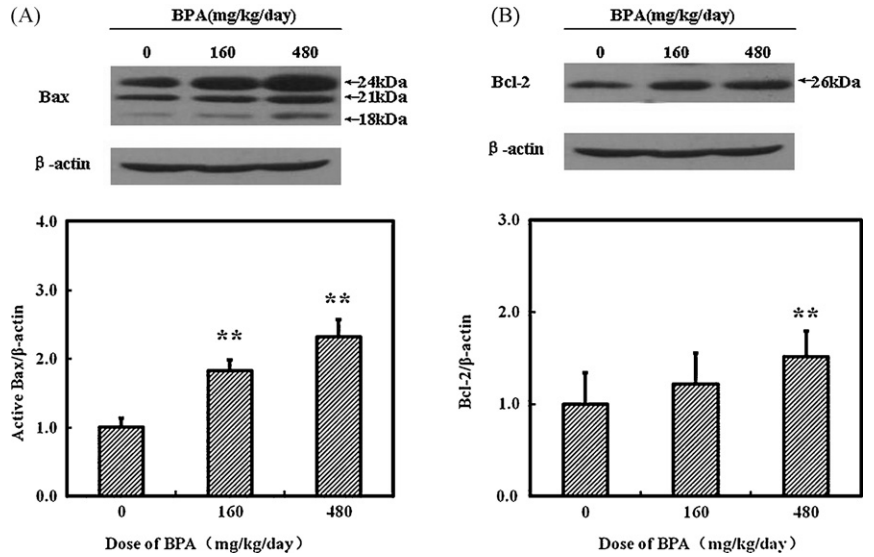

Fig. 6. Effects of pubertal BPA exposure on the expression of Bcl-2 and Bax in testes. The protein expression of Bcl-2 and Bax in testes was measured using immunoblotting. (A) Active Bax (18 kDa); (B) Bcl-2. All data were expressed as means ± SD of six samples from six different mice. $^{**}P < 0.01$ as compared with controls.

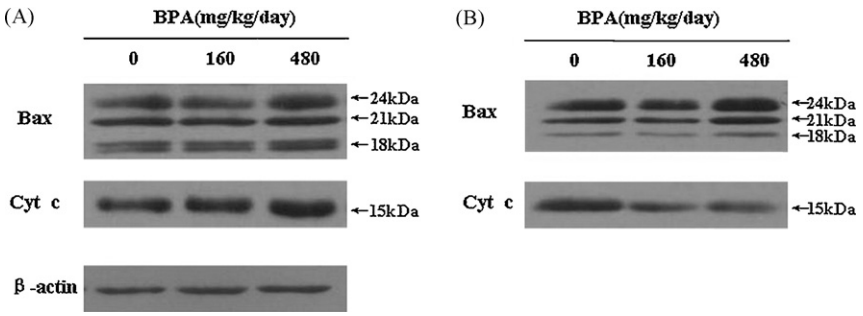

Fig. 7. Effects of pubertal BPA exposure on the distribution of cytochrome c and Bax in mitochondria and cytoplasma. The level of cytochrome c and Bax in mitochondria and cytoplasma was measured using immunoblotting. Cytochrome c and Bax (A) in cytoplasma and (B) in mitochondria.

showed that pubertal BPA exposure significantly reduced the number of Leydig cells in testes and decreased plasma and testicular testosterone levels (Nakamura et al., 2010). Thus, the decrease in testicular testosterone level might contribute, at least partially, to BPA-evoked stage specific germ cell apoptosis in testes.

Several studies have demonstrated that Fas and FasL play an important role in the apoptotic death of germ cells that results from reduced intratesticular testosterone levels or hormone deprivation (Nandi et al., 1999; Pareek et al., 2007). According to an earlier study, diethylstilbestrol, a synthetic estrogen, induces germ cell apoptosis in testes by increasing the expression of spermatogenic cell Fas and FasL (Nair and Shaha, 2003). Several recent studies showed that lindane and p,p′-DDE, two famous endocrine disruptors, induced germ cell apoptosis through Fas/FasL signaling pathway (Shi et al., 2010; Saradha et al., 2009). In the present study, we show that protein level of Fas was significantly increased in testes of mice exposed to BPA during puberty. In addition, pubertal BPA exposure significantly upregulated the expression of FasL in testes in a dose-dependent manner. The level of active caspase-8 and caspase-3 was also increased in testes of mice treated with BPA during puberty. These results are in agreement with a recent study, in which upregulation of Fas and FasL was observed in testes of mice exposed to BPA (Li et al., 2009). These results suggest that Fas/FasL signaling pathway is involved in the process of BPA-induced germ cell apoptosis in testes.

It has been demonstrated that mitochondrial signaling pathway is important for germ cell apoptosis in testes. According to an earlier study, mitochondrial signaling pathway was the key apoptotic pathway for estrogen-induced germ cell apoptosis in testes, in which translocation of cytochrome *c* from the mitochondria to the cytosol were observed in rats treated with diethylstilbestrol (Nair and Shaha, 2003). More and more studies indicate that mitochondrial signaling pathway is also involved in germ cell apoptosis caused by environmental estrogens. A recent study showed that methoxychlor, an endocrine disruptor, resulted in a significant increase in the levels of cytosolic cytochrome c and procaspase-9 as early as 6 h following exposure (Vaithinathan et al., 2010). Additional study found that a significant elevation in the levels of cytosolic cytochrome c with a parallel increase in procaspase-9 were observed as early as 6 h following lindane exposure (Saradha et al., 2009). The present study showed that cytosolic cytochrome c was significantly increased and mitochondrial cytochrome c was significantly decreased, indicating that translocation of cytochrome *c* from the mitochondria to the cytosol in testes of mice exposed to BPA during puberty. Importantly, the present study found that the level of active caspase-9, a marker of mitochondrial apoptotic pathway, was significantly increased in testes of mice exposed to BPA during puberty. These results suggest that BPA induces germ cell apoptosis possibly through mitochondrial apoptotic pathway.

Bcl-2 family play an important role on mitochondrial apoptotic pathway. Bcl-2 is the antiapoptotic member of Bcl-2 family that retard cytochrome c release from mitochondria into cytosol, whereas Bax is the proapoptotic member of Bcl-2 family and counteracts the cytoprotective effect of Bcl-2 by promoting cytochrome c release from mitochondria into cytosol (Dejean et al., 2006; Autret and Martin, 2009). To explore the role of Bcl-2 family in BPA-mediated germ cell apoptosis in testes, the present study measured the effects of pubertal BPA exposure on the expression of Bcl-2 and Bax in testes. Our results showed that pubertal BPA exposure significantly upregulated the expression of Bax in testes in a dose-dependent manner. Importantly, the level of Bax in mitochondrial fractions of testicular lysates was significantly increased in mice exposed to BPA during puberty. Unexpectedly, pubertal BPA exposure did not reduce protein level of the antiapoptotic member Bcl-2 in testes. Actually, the expression of Bcl-2 was slightly upregulated in testes of mice exposed to a high dose of BPA during puberty. However, the ratio of the proapoptotic member Bax vs the antiapoptotic member Bcl-2 was significantly increased in testes of mice exposed to BPA during puberty. Thus, the increased Bax might be involved in the process of BPA-mediated cytochrome c release from mitochondria into cytosol and subsequent activation of caspase-9 and caspase-3.

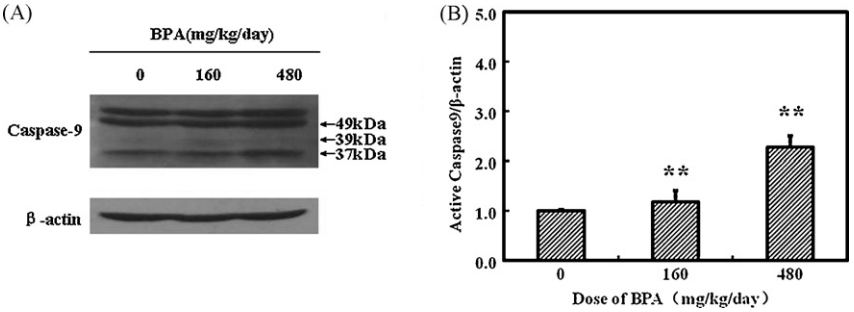

Fig. 8. Effects of pubertal BPA exposure on the level of caspase-9 in testes. The level of active caspase-9 (37 kDa) was measured in testes using immunoblotting. All data were expressed as means ± SD of six samples from six different mice. $^{**}P < 0.01$ as compared with controls.

In summary, the present study investigated the effects of pubertal BPA exposure on germ cell apoptosis in testes. Our results indicate that pubertal BPA exposure induced germ cell apoptosis in testes. Apoptotic germ cells were observed mainly in stages VII–VIII seminiferous tubules in testes. BPA-mediated germ cell apoptosis in testes depends on not only the Fas/FasL signaling but also the mitochondrial pathway.

Conflict of interest statement

There are no conflicts of interest.

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