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Bisphenol AF may cause testosterone reduction by directly affecting testis function in adult male rats

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ABSTRACT

Although in vitro studies have indicated that Bisphenol AF (BPAF) might be a more dangerous endocrine disruptor than Bisphenol A (BPA), no information on reproductive toxicity in animals is available. In this study, the effects of BPAF exposure on the testis and the related mechanisms of toxicity were investigated. Sprague–Dawley (SD) male rats were exposed to BPAF (0, 2, 10, 50 and 200 mg/kg/d) for 14 days. Total cholesterol levels in serum were decreased in rats given a dose of 50 and 200 mg/kg/d. BPAF concentration in the testes increased with increasing doses of BPAF. Reduced serum testosterone and increased luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels were observed in rats in the higher dose groups. Furthermore, BPAF exposure resulted in a dramatic decline in genes and protein involved in cholesterol biosynthesis, transport and steroid biosynthesis. Similarly, the testicular mRNA levels of inhibin B, estrogen receptor (ERα) and luteinizing hormone receptor (LHR) also decreased in rats given a dosage of 200 mg/kg/d BPAF. Together, these data demonstrate that BPAF-induced inhibition of testosterone production primarily resulted from the alteration of genes and proteins in the testosterone biosynthesis pathway.

1. Introduction

Bisphenol AF (BPAF), an analog of Bisphenol A (BPA), is widely used as a crosslinking reagent in the production of fluoropolymers and fluoroelastomers and as a monomer in the production of many polymers, such as polyimides, polyamides, polyesters, polycarbonates, and food-contact polymers (Akahori et al., 2008; Perez et al., 1998). It was estimated that about 10,000–500,000 pounds of BPAF are produced annually in the United States (Stout, 2008). Although there is no annual production information available for China, there are more than forty manufacturing sites.

Though BPAF is widely used, information on its toxicological outcomes, environmental presence and environmental fate are very limited. In a German study, BPAF was detected in about three-fourths of collected surface water and sewage samples, as well as in more than half of the sediment samples (Stout, 2008). Analysis of the structure–activity relationship (SAR) of BPA and its related compounds implied that BPAF might be a more dangerous synthetic estrogenic chemical than BPA due to the substitution of the propane bridge of BPA, which is related to its endocrine-disrupting activity, with a hydrophobic group. This type of substitution has been shown to increase estrogenic activity (Bermudez et al., 2010; Kitamura et al., 2005). Daily subcutaneous injections of 100 mg/kg BPAF to immature female rats for three days led to a 337% increase in uterus size, compared to only a 197% increase when exposed to 200 mg/kg BPA. These findings imply that BPAF is more toxic than BPA (Stout, 2008). An estrogen luciferase reporter assay demonstrated that the estrogen activity of BPAF was about one order of magnitude stronger than that of BPA (Kitamura et al., 2005). Therefore, BPAF was nominated by the U.S. National Institute of Environmental Health Sciences for further study in 2008 due to its potential to interfere with endocrine activity (National Toxicology Program (NTP) 2008). The suspected toxicity and the endocrine-disrupting capabilities of BPAF have raised concerns among environmental scientists.

In vitro studies indicated that BPAF has the ability to bind with both estrogen receptors, ERα and ERβ. The binding affinity of BPAF was approximately 20 times stronger and 48 times stronger than that of BPA as a ligand for ERα and ERβ, respectively (Matsushima et al., 2010). Considerable evidence indicated that BPA exerts its endocrine-disrupting activity by interacting with members of the nuclear steroid receptor family, such as ERα, ERβ, and androgen receptors (ARs) (Wetherill et al., 2007). Moreover, BPA has been reported to reduce daily sperm production in pubertal rats (Herath et al., 2004), suppress serum luteinizing hormone (LH) and testosterone levels as a result of decreased steroidogenic enzymes (Akingbemi et al., 2004; Nakamura et al., 2010), and inhibit the gene levels of ERα with unchanged ERβ levels in the testes (Nakamura

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et al., 2010). Whether BPAF can exert its endocrine-disrupting activity and cause testicular toxicity via a mechanism involving ERs located in the testes is unknown, and no research on this topic has been conducted to date.

Testosterone is synthesized by Leydig cells from cholesterol, which plays a key role in the development of the male reproductive system and spermatogenesis in the testes (Ye et al., 2011). Previous studies have implied that endocrine-disrupting chemicals could act to disrupt androgen synthesis and secretion by directly inhibiting enzymes in the testosterone biosynthesis pathway or by indirectly altering pituitary function (Nakamura et al., 2010; Shi et al., 2007). Thus, the aim of this study is to determine whether BPAF exposure will produce adverse effects on testosterone production and to further elucidate the mechanism of BPAF toxicity in testes.

In this study, adult male rats were dosed via oral gavage with 0, 2, 10, 50 and 200 mg/kg/d BPAF for 14 days. The toxicity of BPAF was evaluated by testicular BPAF concentration, serum hormone levels of LH, FSH, and testosterone, as well as serum total cholesterol levels. In addition, the mRNA levels of nuclear steroid receptors were detected. The involvement of steroidogenesis in the testes was also investigated at both gene and protein levels.

2. Materials and methods

2.1. Animals

Male Sprague–Dawley (SD) rats aged 7 weeks were obtained from the Academy of Military Medical Sciences, Beijing, China. Six animals were assigned to each treatment and control group. Animals were housed one per metabolic cage and maintained in a mass air displacement room with a 12-h light–dark cycle at 20–26 °C with a relative humidity of 30–70%. Animals had access to food and water ad libitum. All rats were acclimatized for one week before experiments were begun.

2.2. Chemicals and treatments

BPAF (CAS No. B0945, 99% purity) was purchased from Tokyo Chemical Industry (TCI, Tokyo, Japan). To achieve different doses, BPAF was dissolved in corn oil vehicle including 4% ethanol, which was prepared fresh each day. BPAF was administered orally via gavage to rats in the treatment group for 14 days at doses of 2, 10, 50 and 200 mg/kg body weight/day in a volume of 5 ml/kg of body weight. Control rats were treated similarly with the vehicle alone. During the experimental days, the rats were weighed each day to administer the dose per kilogram of body weight. The chosen doses were based on a study in which the oral 50% lethal dose (LD50) in rats exposed acutely was up to 3400 mg BPAF/kg (Halocarbon, 2007). After 24 h of the last BPAF dosing, all rats were weighed and euthanized by decapitation. Blood was collected and centrifuged at 3000 rpm at 4 °C for 15 min. Serum was stored at −80 °C until analysis. Testes were immediately isolated, weighed, snap frozen in liquid nitrogen and stored at −80 °C for RNA isolation and protein extraction.

2.3. Serum total cholesterol level

Total cholesterol (TCHO) in serum was measured with a commercial TCHO kit according to the manufacturer's recommendations (Beijing Applygen Limited Company, Beijing, China). Total cholesterol was measured using a Bio-Rad SmartSpec 3000 spectrophotometer (Bio-Rad, California, USA) at 550 nm.

2.4. BPAF concentration in testes

Testes were extracted by acetonitrile and then purified by solid phase extraction (SPE) cartridges for protein and lipid elimination. The concentration of BPAF in testes was determined with an Acquity ultra performance liquid chromatography (UPLC) coupled to a Xevo triple quadrupole mass spectrometer (Waters, Milford, MA, USA).

2.5. Serum hormone levels

Because the FSH and LH levels in some rats were lower than the detection limit of the ELISA kits, these two hormones were measured by radioimmunoassay (RIA) using commercial kits from the Beijing North Institute of Biological Technology, China. Concentrations of serum testosterone were measured by ELISA using commercial human ELISA kits (Roche, Mannheim, Germany). The assay detection limit

Table 1
Sequences of primers used for real-time RT-PCR amplification.

Target gene	GenBank accession no.	Product length (bp)	Primer sequences	T_m (°C)
SR-B1	AY451993	156	Sense: 5′-ACAGGTCCCAGGGCTCAG-3′ Anti-sense: 5′-CGTGCGGTTCATAAAGG-3′	57.0
StAR	NM_031558	111	Sense: 5′-GGGCATACTCAACAACCAG-3′ Anti-sense: 5′-ACCTCCAGTCGGAACACC-3′	57.0
P450scc	J05156.1	125	Sense: 5′-AGTATCCGTGATGTGGGG-3′ Anti-sense: 5′-CATACAGTGTCGCCTTTTCT-3′	60.0
3β-HSD	M38178	145	Sense: 5′-TGTGCCAGCCTTCATCTAC-3′ Anti-sense: 5′-CTTCTCGGCCATCCTTTT-3′	53.0
CYP17a	NM_012753	142	Sense: 5′-GCAGAGTTACTTGCCCTTCGG-3′ Anti-sense: 5′-CAGGCGGGGCAGTTGTTTAT-3′	60.0
17β-HSD	NM_054007.1	82	Sense: 5′-TGTGGCTGCCTTGCTCAT-3′ Antisense: 5′-TTTGGGTGGTGCTGCTGT-3′	60.0
ER-α	NM_012689.1	431	Sense: 5′-GCCAAGGAGACTCGCTAC-3′ Anti-sense: 5′-GCATCCAATAAGGCACTG-3′	60.0
ER-β	NM_012754	152	Sense: 5′-CTCCTTTAGCGACCCATTG-3′ Anti-sense: 5′-AACAGGGCTGGCACAACT-3′	60
AR	NM_012502	106	Sense: 5′-GCCTCTGGCCGAATGCA-3′ Anti-sense: 5′-CAACCCTTTGGCGTAACCT-3′	55.0
LHR	NM_012978	130	Sense: 5′-CATTCAATGGGACGACTCTA-3′ Anti-sense: 5′-GCCTGCAATTTGGTGGA-3′	57.0
Inhibin B	XM_344130	186	Sense: 5′-ACCTGAAACTGCTCCCCTAT-3′ Anti-sense: 5′-TCGCCTCGCTCAAACAA-3′	55.0
MIS	NM_012902	245	Sense: 5′-GGGAGCAAGCCCTGTTAG-3′ Anti-sense: 5′-GCGGGAATCAGAGCCAAA-3′	60.0
HMGR	NM_013134.2	83	Sense: 5′-TGTCAAGACTTTTCCGTATG-3′ Anti-sense: 5′-GATAGTAAGTGTCACCGTTCC-3′	60.0
SREBP-1c	AF286470.2	125	Sense: 5′-GGGACTACAGGCTGAGAAA-3′ Anti-sense: 5′-CCAGGTTAGAAGCAACAAG-3′	60.0
β-Actin	NM_031144	134	Sense: 5′-TCGTGCGTGACATTAAAGAG-3′ Anti-sense: 5′-ATTGCCGATAGTGATGACCT-3′	60.0