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Influence of Occupational Exposure to Bisphenol A on the Sex Hormones of Male Epoxy Resin Painters

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Abstract

Epidemiological studies demonstrate an adverse effect of several environmental and occupational exposures on male sex hormone. Bisphenol A (BPA) is a weak estrogen and a widely used industrial chemical. Epoxy resin painters may be continuously exposed to BPA at high concentrations. The effect of occupational exposure of BPA on male reproduction was examined by measuring the urinary BPA, testosterone and gonadotropic hormones of epoxy resin painters in the shipyard. The painters had significantly higher concentrations of urinary BPA (2.61 ± 1.08 μg/g creatinine) than controls (1.38 ± 0.5 9 μg/g creatinine). In serum, the testosterone level of painters was significantly decreased but the luteinizing hormone (LH) and follicle-stimulating hormone (FSH) levels of painters were significantly higher than controls. Occupational exposure to BPA influences testosterone and gonadotropic hormones in male workers.

Keywords: Bisphenol A, Occupational exposure, Epoxy resin painters, Testosterone, Gonadotropic hormones

Bisphenol A [2,2-bis(4-hydroxyphenyl) propane, BPA] is a monomer used in the production of polycarbonates and epoxy resins. The amount of BPA used in the world is estimated at 3.7 million tons (2004) and is predicted to increase by 6 to 7% every year[1]. Because products made from BPA are used widely in daily life, the general population's exposure is estimated to be 9 μg/kg/day of BPA[2]. Krishnan *et al*.[3] suggested weak estrogenic activity of BPA which has been demonstrated in a variety of assays. Cell proliferation is induced within the MCF-7 cells and stimulates the release of prolactin from the pituitary GH_3 cells[4]. The estrogenic potency of BPA is 1/15,000 lower than 17β-estradiol and 1/10 lower than 4-nonylphenol[5,6]. The estrogenecity of BPA *in vivo* is determined by the route of administration and oral bioavailability of BPA is low, and thus BPA glucuronide without estrogenecity is formed intensively in the liver[7-9]. The effect of BPA on male reproduction remains questionable. However, BPA may possibly combine with the estrogen receptor (ER) and induce an ER-mediated gene expression. LH and testosterone levels have been observed to be decreased in rats exposed to BPA[10]. Endogenous BPA suppressed secretion of FSH in men but the clinical significance was unclear[11]. Although several results show low amounts of BPA could cause adverse effects *in vivo* and *in vitro*, the risk assessment of human exposure to BPA is difficult to study. However, Völkel *et al*.[2] suggested the efficient glucuronidation of BPA and the rapid excretion of the formed glucuronide result in a low body burden of the estrogenic BPA in humans following oral absorption of low doses.

In the ship building industry, epoxy resin paint is used to prevent oxidation of the sheet steel. There is approximately 10 to 30% of BPA contained in the epoxy paint, therefore painters are potentially exposed to several organic solvents and estrogenic BPA. However, there is not enough interest in this type of occupational exposure and effect on the male reproduction. This study examined the male painter's urinary BPA to estimate the occupational exposure level and defined the effect of BPA on the gonadotropic hormones and testosterone.

Characteristics of Subjects

Epoxy resin painters were recruited as the exposure

group and a similar aged non-painter group was selected for the control group. Painters and controls were less than 52 yrs of age and the average age was 42.8 ± 5.8 yrs and 40.1 ± 7.1 yrs, respectively.

Approximately 80% of the painters smoked and 68% of the controls smoked. Alcohol was consumed by 88% of the painters and 76% of the controls. Painters had worked at their job for 16.1 ± 4.7 yrs and controls for 10.4 ± 6.4 yrs.

Urinary BPA and Serum Hormone Levels

There was a significant difference between the urinary BPA level of painters and non-painters. Painters had a mean of 2.61 ± 1.08 μg/g creatinine (95% Confidential Index (C.I); 2.17-3.06) and non-painters had a mean of 1.38 ± 0.59 μg/g creatinine (95% C.I; 1.11-1.60). Although all of the subject's testosterone, LH and FSH concentrations were within the reference value, there was a significant difference between the 2 groups. Painters had a testosterone level of 3.51 ng/mL, which was significantly lower than the non-painters level of 5.18 ng/mL. The LH level between the painters and non-painters also differed. The painters had a significantly higher level at 5.34 ± 1.68 IU/L compared to the non-painters (3.16 ± 1.40 IU/L). Additionally, the painters had a FSH level of 7.68 ± 2.54 IU/L, significantly higher than the non-painters FSH level of 5.33 ± 2.11 IU/L (Table 1). Table 2 shows the correlation results between urinary BPA, gonadotropic hormones, testosterone and age. As age increased, testosterone was decreased and urinary BPA, LH and FSH were increased. However, the 2 correlations were not significant. LH was increased significantly with increasing urinary BPA. There was no significant correlation of testosterone and LH or FSH.

Table 1. Concentrations of urinary BPA and serum hormones.

	Painter	Controls	*P*
BPA (μg/g creatinine)	2.61 ± 1.08	1.38 ± 0.59	0.000
Testosterone (ng/mL)	3.51 ± 0.74	5.18 ± 1.04	0.000
LH (IU/L)	5.34 ± 1.68	3.16 ± 1.40	0.000
FSH (IU/L)	7.68 ± 2.54	5.53 ± 2.11	0.002

Unit: geometric mean ± geometric standard deviation, painters who used epoxy resin paint (n=25) and non-painters (n=25). All variables showed a significant difference between the 2 groups.

Table 2. Correlation coefficients between urinary BPA, serum hormones, and age.

	Testosterone	LH	FSH	Age
BPA (μg/g creatinine)	−0.189	0.482*	0.184	0.236
Testosterone (ng/mL)		−0.229	−0.238	−0.079
LH (IU/L)			0.390*	0.080
FSH (IU/L)				0.201

*$P < 0.05$

Multiple Regression Analysis

Multiple regression analysis was performed between the hormones and the general characteristics (age, smoking and drinking habits), occupational characteristics (exposure index, work duration) and urinary BPA. Occupational characteristics were a significant variable that decreased the level of testosterone. Urinary BPA was a significant variable for the LH level and the exposure index of organic solvents was a significant variable that affected the FSH level (Table 3).

Discussion

In this study, the urinary BPA, serum gonadotropic hormones and testosterone of workers who used epoxy resin paints containing BPA in the shipyard were examined. Workers who spend a significant amount of time at the facilities are potentially exposed to harmful chemicals, which could cause adverse effects on sex hormone. However, most of the workers are not aware that occupational exposure could be harmful for reproduction.

Carlson *et al.*[13] reported there was a 50% decrease in the sperm count over the past 50 years (1940 to 1990). Exposure to certain chemicals and physical factors may cause reversible or irreversible damage

Table 3. Multiple regression analysis of testosterone, LH and FSH.

	Testosterone		LH		FSH	
	β	*P*	β	*P*	β	*P*
BPA (μg/g creatinine)	−0.049	0.754	0.487	0.040	−0.057	0.855
Work duration (yr)	−0.538	0.013	−0.088	0.615	0.483	0.007
Exposure index	−0.332	0.041	0.221	0.174	−0.083	0.615

β (standardized coefficient) was adjusted for age, as well as smoking and alcohol habits. Adjusted R^2 of the testosterone model, LH model, and FSH model was 0.401, 0.378, and 0.258, respectively.

to fertility. However there are only a few chemicals proven to be toxic to human reproduction[14]. Although occupational organic solvent exposures have been inconsistently associated with effects of reproductive hormone levels in men, exposure to specific solvents is a risk factor for the male fertility.

Effects of BPA on male reproduction have been controversial. Exposure of rodent fetuses to BPA produced postnatal estrogenic effects, which reduced daily sperm production and increased prostate gland weight in males[15]. Oral administration of BPA to pregnant mice resulted in a permanent increase in prostate size and expression of prostate androgen receptors. BPA also caused a decrease in the size of the epididymis[16,17]. After puberty, BPA suppressed serum testosterone levels in male rats which led to a decrease in the sperm count[18]. In studies of exposure to lower BPA levels, an inhibitory effect on testicular steroidogenesis, aromatase gene expression and 17β-estradiol biosynthesis was found. In addition, serum LH and testosterone's secretion was suppressed[10].

In this study, the testosterone level in the painters decreased significantly but LH and FSH increased significantly. As the BPA exposure level increased, testosterone level decreased, consistent with results from previous studies. Several studies showed a suppressed LH level, although in the present study, increased LH levels were observed in painters. The research design of this study was cross-sectional and there were limits such as selection bias and unclearness of the cause and effect relationship. However, Tohei *et al*.[19] suggested that the reduction in the negative feedback regulation by testosterone may cause an increase in the LH level of male rats exposed to BPA. The painters had a significantly higher FSH level than the controls. Evans *et al*.[20] observed that FSH remained relatively constant but Hanaoka *et al*.[11] reported the secretion of FSH in the BPA diglycidyl ether was suppressed in the exposure group. In this study, the painters used epoxy resin paint that contained several organic solvents. The significant associations between FSH levels and solvent exposure indices suggest the potentiality for adverse effects of exposure to occupational solvents on the reproductive function in men[21]. In addition, Mørck *et al*.[22] showed chronic toluene exposure was associated with increased FSH concentrations. The painter's exposure indices did not exceed the exposure limits but their mean work duration was 16 yrs, thus the chronic exposure to these solvents has possibly affected the FSH secretion.

BPA glucuronide is a very suitable marker to measure the exposure of BPA because it exists at high levels in urine. Meanwhile, the measurement of free BPA is used as a marker related to potential estrogenic effects[2]. Therefore, to assess BPA exposure, the total urinary BPA was measured by using enzyme (glucuronidase/sulfatase) hydrolysis.

In this study, the urinary BPA concentration of painters was 2.61 μg/g creatinine, significanly higher than the non-painters BPA concentration of 1.38 μg/g creatinine. Urinary BPA of epoxy resin sprayers who were exposed to BPA diglycidyl ether in the machine plant was 2.14 μg/g creatinine and that of control groups was 1.05 μg/g creatinine[11]. According to the Third National Health and Nutrition Examination survey, healthy adults (n=394) had a urinary BPA level of 1.36 μg/g creatinine[23]. Although the study sample and research methods differed from each other, the previous experiments showed similar results.

The BPA exposure levels could not be quantified but were confirmed from the MSDS to contain approximately 10 to 30% of epoxy resin. Occupational exposure to BPA could influence testosterone and gonadotropic hormones. However, whether the result is from exposure to BPA alone or is due to the work conditions or exposure to other organic solvents is unclear. In conclusion, work duration and exposure index have a significant effect on the secretion of testosterone, concentration of urinary BPA on LH, and exposure index on FSH. The results from this study provide a basis for further research where occupational exposure to single BPA can be properly evaluated by enlarging the sample size.

Materials and Methods

Subjects

Twenty-five epoxy resin painters working at a shipyard were recruited. The paint used was confirmed as containing 10 to 30% BPA based on the Material Safety Health Sheet (MSDS). A control group was selected consisting of 25 non-painters. Along with sampling, the study participants were asked their age, sex, work duration, work department, job description and drinking and smoking habits through a self-reported questionnaire. All subjects gave their written informed consent.

Analysis of Urinary BPA

Matsumoto's method[12] was modified for analysis of the total conjugated form plus the free form of urinary BPA. Urine (500 μL) was buffered with 200 μL 50 mM sodium acetate buffer (pH 4.9 to 5.0 by H_3PO_4) in a 20 mL vial covered with Teflon and hydrolyzed enzymatically with β-glucurodidase/sulfatase for 3 hrs at 37°C in a shaking water bath. After hydrolysis, 100 μL of 1 N HCl was added and the hydrolysate

was extracted with 5 mL tert-butyl methyl ether that contained the internal standard bisphenol F for 30 min at 200 rpm. After centrifugation (at 500 rpm for 5 min), 4 mL l of the supernatant was transferred to a new tube and dried. The residue was dissolved with 200 μL of 40% acetonitrile in water and 60 μL of solution was used for high performance liquid chromatography (HPLC). Urinary creatinine was determined using Jaffe's picric acid method kit from Merck (Darmstadt, Germany). All samples were corrected with urinary creatinine and the liquid-liquid extraction was corrected with the bisphenol F recovery rate.

Chromatographic Equipment and Conditions

To analyze the urinary BPA, the Empower Pro software and Waters HPLC system (Waters, MA, USA) consisting of a 2695 Alliance separation module and a 2475 Multi λ Fluorescence detector were used. The separation of hydrolysate was performed on a Waters XTerra RP18 analytical column (5 μm, 4.6 × 150 mm), and the column heater temperature was set at 35˚C. The mobile phase used for the isocratic elution of hydrolysate was composed of 75% 5 mM sodium acetate and 25% acetonitrile. Fluorescence excitation was at 230 nm and emission was at 310 nm.

Sex Hormones

The levels of LH, FSH and testosterone were measured. The time of blood collection was limited from 0900 to 1200 hrs in consideration of the diurnal variation. The blood was centrifuged within 30 min after collection and stored at −20˚C until analyzed. LH and FSH were analyzed by immunoradiometric assay and testosterone was analyzed by radioimmunoassay. LH and FSH were analyzed by the Coat A count LH IRMA, FSH IRMA kit (DPC, USA) and r-counter (COBRA 5010 II, Packard, USA). Testosterone was analyzed by a COTA-A-COUNT Testosterone kit (DPC, USA) and r-counter (COBRA 5010 Quantum, Packard, USA).

Statistical Analysis

All data was analyzed using SPSS (Version 12.0E, SPSS Institute Inc., Cary, NC, USA). A t-test and a X^2 were used to compare the general characteristics of the subjects. The t-test was used to study the differences of urinary BPA, testosterone, LH and FSH between painters and controls. The correlation was used to calculate the relationship between urinary BPA levels and the hormones. To adjust for age, and smoking and alcohol habits, a multiple linear regression analysis was used to study relationships between the explanatory variables and the hormones. The level of significance was 0.05.

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