Human Reproduction Vol.20, No.8 pp. 2325–2329, 2005 doi:10.1093/humrep/deh888
Advance Access publication April 7, 2005

Exposure to bisphenol A is associated with recurrent miscarriage

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BACKGROUND: Little is known about the influence of high exposure to bisphenol A on recurrent miscarriage and immunoendocrine abnormalities. METHODS: Serum bisphenol A, antiphospholipid antibodies (aPLs), antinuclear antibodies (ANAs), natural killer cell (NK) activity, prolactin, progesterone, thyroid-stimulating hormone (TSH) and free T4 were examined in 45 patients with a history of three or more (3–11) consecutive first-trimester miscarriages and 32 healthy women with no history of live birth and infertility. Subsequent pregnancy outcome and embryonic karyotype of abortuses were examined prospectively. RESULTS: The mean ± SD values for bisphenol A in patients were 2.59 ± 5.23 ng/ml, significantly higher than the 0.77 ± 0.38 ng/ml found for control women ($P = 0.024$). High exposure to bisphenol A was associated with the presence of ANAs but not hypothyroidism, hyperprolactinaemia, luteal phase defects, NK cell activity or aPLs. A high level of bisphenol A in itself did not predict subsequent miscarriage. CONCLUSION: Exposure to bisphenol A is associated with recurrent miscarriage.

Key words: aneuploidy/antinuclear antibody/bisphenol A/embryonic karyotype/recurrent miscarriage

Introduction

Many chemical compounds introduced into the environment by human activity are known to influence the endocrine system of various animals and humans. Kogevinas (2001) has reported dioxin to cause cancer and endometriosis, and Longnecker *et al.* (2001) recently suggested that DDT [1,1,1,-trichloro-2,2-bis (*p*-chlorophenyl) ethane] use increases preterm birth and small for gestational age babies in humans. Endocrine disruptors such as dioxin are well established as reproductive toxicants. However, it is a matter of controversy whether they might be linked with first-trimester spontaneous abortion because there is limited information concerning this issue, especially recurrent miscarriages (Leoni *et al.*, 1989). We previously studied the serum concentration of polychlorobiphenyls (PCBs), hexachlorobenzene (HCB) and the DDT metabolite DDE [1,1-dichloro-2,2-bis (*p*-chlorophenyl) ethylene] and found no association with recurrent miscarriage (Sugiura-Ogasawara *et al.*, 2003).

Bisphenol A [BPA; 2, 2-bis(4-hydroxyphenyl) propane] is well known as an endocrine disruptor having estrogen activity. About 350 000 t/year is produced for use in polycarbonate plastics, epoxy resins, dental sealants and food package lacquers to coat food cans in Japan (Biles *et al.*, 1997; Olea *et al.*, 1996; Biles *et al*., 1999). We are surrounded by materials made from BPA and exposed to this environmental contaminant worldwide. BPA administered to pregnant mice is known to be transferred to fetuses and to alter post-natal development and sexual maturity (Howdeshell *et al.*, 1999). Recently, Hunt *et al.* (2003) demonstrated that daily oral dosing with the compound causes meiotic aneuploidy in the female mouse.

About 40–70% of sporadic spontaneous abortions are linked to chromosomal abnormalities of the conceptus, especially aneuploidy (Creasy, 1988; Ogasawara *et al.*, 2000). No unequivocal cause is currently available for more than half of the cases suffering recurrent miscarriages (Hertz-Picciotto and Samuels, 1998). An abnormal embryonal karyotype of the conceptus has been found in 10–50% of recurrent miscarriage cases (Stern *et al.*, 1996; Ogasawara *et al.*, 2000; Christiansen *et al.*, 2002; Stephenson *et al.*, 2002) and meiotic aneuploidy in 29.9% in one series of such cases (Ogasawara *et al.*, 2000). We hypothesed that elevated exposure to BPA might induce meiotic aneuploidy in females and cause recurrent miscarriages. Thus, we conducted a prospective study. Also, we studied the association between serum levels of BPA and endocrine and immunological abnormalities in a series of recurrent miscarriage patients.

Methods

Patients

We studied 45 patients with a history of three or more (3–11) consecutive first-trimester miscarriages. Hysterosalpingography, chromosome analysis for both partners, immunological tests for parameters such as antinuclear antibodies (ANAs), antiphospholipid antibodies (aPLs) and natural killer (NK) cell activity, and blood tests for hypothyroidism, diabetes mellitus and hyperprolactinaemia were performed for all cases before subsequent pregnancy.

Cases with any uterine anomaly or chromosome abnormality in either partner were excluded from the study. None had a history of live birth. All were seen at Nagoya City University Hospital between August 2001 and December 2002. Fasting blood samples were taken at the same time.

Thirty- two healthy non-pregnant women with no history of live birth, infertility and miscarriage were examined as controls. Patients and controls lived in Nagoya City and the surrounding neighbourhood. None was from specific geographical areas or taking oral contraceptives. Half of the patients were housewives and the others had many different occupations. Healthy volunteers were staff such as doctors of Obstetrics and Gynaecology and other departments, nurses and secretaries in Nagoya City University Medical School. Data regarding place of residence and occupation for patients and controls is shown in Table I. Informed consent approved by the Institutional Review Board was obtained from all subjects.

Mean ± SD values for serum concentrations of BPA in patients were compared statistically with data for controls. Correlations with the presence of ANAs, aPLs, hypothyroidism, luteal phase defects and hyperprolactinaemia were also assessed. Correlations among serum progesterone, prolactin and NK cell activity were examined by Pearson's correlation coefficient.

Subsequent pregnancy outcome was examined prospectively. Ultrasonography was performed twice a week during pregnancy, and dilation and curettage were undertaken when a miscarriage was diagnosed. The karyotype of each aborted conceptus was ascertained using a standard G-banding technique.

Table I. Demographic characteristics and bisphenol A values in patients and control women

	Patients (n = 45)	Controls (n = 32)	P-values
Age (years)	31.6 ± 4.4	32.0 ± 4.8	NS
No. of previous miscarriages	4.0 ± 1.7 0		
BMI	20.9 ± 2.7	20.81 ± 2.25	NS
Place of residence	Nagoya	Nagoya	
Occupation			
	Housewives 20	Medical co-workers	
	Office workers 17 Teacher 3 Medical co-workers 3 Gardener 1 Worker in industry 1		
Bisphenol A			
Mean (SD)	2.59 ± 5.23	0.77 ± 0.38	0.024
Range	0.22–29.43	0.20–1.58	
Median	0.71	0.705	
Quartiles	1.403	0.415	

Values are means ± SD.
BMI = body mass index (kg/m^2).

Serum BPA levels were compared between cases with successful pregnancy and miscarriages with a normal and an abnormal embryonal karyotype.

Antinuclear antibodies, antiphospholipid antibodies, natural killer cell activity, TSH, free T4, progesterone and prolactin measurement

Blood samples were taken 5–9 days after ovulation in at least two cycles. For progesterone and prolactin analysis, they were collected at least 3 months after their last abortion and before they conceived again.

ANAs were assessed by indirect immunofluorescence on Hep-2 cell slides. aPLs were measured by lupus anticoagulant using five times diluted aPTT regents and β2-glycoprotein I-dependent anticardiolipin antibodies by enzyme-linked immunosorbent assay (ELISA) methods (Ogasawara *et al.*, 1996b). NK cell activity was measured by a chromium-51 release cytotoxicity assay, with K562 human myeloid leukaemia cells as the targets.

Progesterone levels were determined by radioimmunoassay, using reagents supplied by the Diagnostic Products Corporation (Los Angeles, CA). A midluteal phase single serum progesterone level <10 ng/ml was used as the criterion for a luteal phase defect. Prolactin was measured by immunoradiometric assay with a kit supplied by Daiichi Radioisotope Laboratories, Ltd. (Tokyo, Japan).

Bisphenol A ELISA measurement

Serial samples were collected from individuals at Nagoya City University Hospital and kept frozen at −20°C until analysis. Briefly, goat anti-rabbit IgG was added to 96-well microtitre plates and, after standing for 2 h at room temperature, the plates were then blocked with 25% Block Ace and stored at 4°C until use. Aliquots of 50 μl of standard antigen (0.5–5000 ng/ml) or samples, BPA-mono-glutanyl–horseradish peroxidase (HRP) and rabbit anti-BPA serum were added in duplicate and the wells were incubated for 2 h at room temperature. Phenylenediamine solution was added and the absorbance was measured at 492 nm (Kodaira *et al.*, 2000).

Statistical analysis

Differences in group values were analysed using Stat view and DA stats with an Apple Macintosh computer. A significance level of $P < 0.05$ was applied for all tests. We used Welch's test to compare BPA levels between patients and controls because the distribution of the two groups might have differed. The Mann–Whitney test was employed for comparisons of BPA levels between patients who had successful pregnancies and patients who miscarried again with and without an abnormal embryonal karyotype and between those with and without hypothyroidism, aPLs, ANAs, luteal phase defect or hyperprolactinaemia. Correlations were analysed with reference to Pearson's correlation coefficients.

Results

The 45 study patients had a mean (± SD) age of 31.6 ± 4.4 years and 4.0 ± 1.7 previous miscarriages (range, 3–11 miscarriages). The 32 controls were 32.0 ± 4.8 years of age (Table I).

The mean ± SD values for BPA in patients were 2.59 ± 5.23, significantly higher than the 0.77 ± 0.38 found for control women (P = 0.024). No linkage was evident with the body mass index (kg/m^2).

Of the 45 patients, eight (15.6%) had hypothyroidism, and six (13.3%) and 10 (22.2%) patients had aPLs and ANAa,

Table II. Bisphenol A values in patients with and without hypothyroidism, antiphospholipid antibodies, antinuclear antibodies, luteal phase defect and hyperprolactinaemia

	Positive	Negative	*P*-value (Mann–Whitney U-test)
Hyperthyroidism			
n	8 (17.8%)	37	
Mean (SD)	2.986 (3.045)	2.505 (5.693)	
Range	0.29–8.37	0.22–29.43	
Median	1.93	0.71	NS
Quartiles	4.645	0.82	
aPL			
n	6 (13.3%)	39	
Mean (SD)	2.065 (3.595)	2.672 (5.541)	
Range	0.22–9.37	0.25–29.43	
Median	0.675	0.74	NS
Quartiles	0.87	1.618	
ANA			
n	10 (22.2%)	35	
Mean (SD)	7.382 (9.761)	1.222 (1.54)	
Range	0.25–29.43	0.22–6.48	
Median	2.28	0.6	0.0252
Quartiles	8.58	0.68	
LPD			
n	9 (20.0%)	36	
Mean (SD)	1.896 (2.08)	2.765 (5.839)	
Range	0.25–6.48	0.22–29.43	
Median	0.64	0.725	NS
Quartiles	2.445	0.875	
Hyperprolactinaemia			
n	5 (11.1%)	40	
Mean (SD)	0.724 (0.309)	2.824 (5.577)	
Range	0.29–1.16	0.22–29.43	
Median	0.71	0.725	NS
Quartiles	0.277	1.81	

aPL = antiphospholipid antibody; ANA = antinuclear antibody; LPD = luteal phase defect.

respectively (Table II). Five (11.1%) and nine (20.5%) demonstrated hyperprolactinaemia and a luteal phase defect. No linkage with NK cell activity, progesterone and prolactin level was evident.

There were no differences in mean values for BPA between patients with and without hypothyroidism, hyperprolactinaemia, a luteal phase defect and the presence of aPLs. However, ANA-positive patients had significantly higher BPA levels than their ANA-negative counterparts ($P = 0.025$).

A total of 35 patients became pregnant subsequently and 17 (48.6%) of these miscarried again. One suffered an ectopic pregnancy and thus there were 17 live births. The BPA level of patients who miscarried subsequently (mean ± SD, 4.39 ± 8.08; range, 0.28–29.43; median, 0.71; quartiles, 3.055) tended to be higher, albeit without significance, than that of patients whose subsequent pregnancy was successful (mean ± SD, 1.22 ± 1.07; range, 0.22–3.85; median, 0.91; quartiles, 1.28).

Thirteen karyotypes of the miscarried conceptus could be analysed. Four were abnormal: 47, XX, + 7, 47, XY, + 9, 47, XY, + 10 and 47, XY, +13. The BPA levels of cases with abnormal embryonal karyotypes were 0.66, 19.09, 0.69 and 29.43, respectively (mean ± SD, 12.47 ± 14.26). The individual BPA levels in each group (patients versus controls, patients who had successful pregnancies versus patients who miscarried again, with an abnormal embryonal karyotype versus a normal embryonal karyotype) are shown in Figure 1.

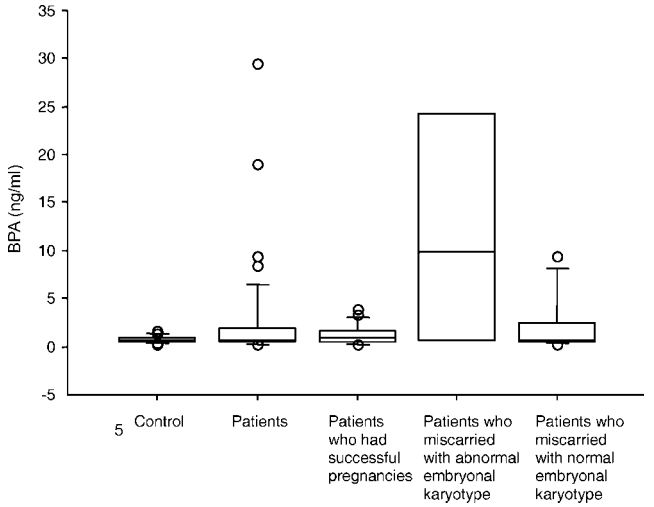

Figure 1. The mean ± SD values for BPA in 45 patients were significantly higher than that in 32 control women. The BPA level of 17 patients who miscarried subsequently tended to be higher, albeit without significance, than that of 17 patients whose subsequent pregnancy was successful.

Discussion

The present study provided the first concrete evidence that high exposure to BPA may be associated with recurrent miscarriage, especially with ANA-positive patients.

It has been a matter of controversy whether chemical compounds are linked with first-trimester spontaneous abortion, and our previous study of serum concentrations of PCBs, HCB and the DDT metabolite DDE found no association between exposure and recurrent miscarriage (Leoni *et al.*, 1989; Sugiura-Ogasawara *et al.*, 2003).

BPA is well known as a chemical compound having 0.00025 times the estrogen activity of estradiol, binding to the estrogen receptor. Steinmetz *et al.* (1997) reported that BPA has 0.0001–0.0005 times the estrogen activity *in vitro*, but with an increase of 0.05 times in terms of stimulating prolactin in rats. BPA may be more potent *in vivo* than *in vitro*. Honma *et al.* (2002) reported that low dose exposure to BPA caused earlier vaginal opening in female mice. Many reports have been published concerning its influence on the female mammalian reproductive system (Ashby and Tinwell, 1998; Milligan *et al.*, 1998; Hiroi *et al.*, 1999; Howdeshell *et al.*, 1999; Takai *et al.*, 2000).

Hunt *et al.* (2003) demonstrated that daily oral dosing exposure causes meiotic aneuploidy in the female mouse (Hunt *et al.*, 2003). They reported a sudden, spontaneous increase in meiotic disturbance, including aneuploidy, in studies of oocytes from control female mice in their laboratory, coinciding with accidental exposure of the animals to an environmental source of BPA. They identified damaged caging material as the source, as they were able to

recapitulate the meiotic abnormalities by intentionally damaging cages and water bottles. In further studies, they administered daily oral doses of BPA to test directly the hypothesis that low levels of BPA disrupt female meiosis. They concluded that the meiotic effects were dose dependent and inducible by environmentally relevant doses. BPA has been found in blood of non-pregnant women at a concentration of 2.0 ± 0.8 ng/ml and in human follicular fluid at a concentration of 2.4 ± 0.8 ng/ml by ELISA methods (Ikezuki *et al.*, 2002). The results further indicated that the oocyte and its meiotic spindle can provide a sensitive assay system for the study of reproductive toxins. Ikezuki *et al.* also showed a significant linear correlation between BPA values obtained by conventional reverse-phase high-performance liquid chromatography (HPLC) and ELISA data (Kodaira *et al.*, 2000; Ikezuki *et al.*, 2002). The BPA values of non-pregnant women in the previous study tended to be slightly higher than in the present study.

About 40–70% of sporadic spontaneous abortions appear to be caused by chromosomal abnormalities of the conceptus, and aneuploidy is especially important (Creasy, 1988; Ogasawara *et al.*, 2000). An abnormal embryonal karyotype of the conceptus has been found in 10–50% of recurrent miscarriage cases (Stern *et al.*, 1996; Ogasawara *et al.*, 2000; Christiansen *et al.*, 2002; Stephenson *et al.*, 2002) and meiotic aneuploidy in 29.9% in one series of such cases (Ogasawara *et al.*, 2000). Both miscarriage and the aneuploidy rate depend on women's age. No unequivocal cause is currently available for more than half of the cases with recurrent miscarriages and, in recurrent aborters, the miscarriage rate increases and abnormal embryonal karyotype rate decreases with the number of previous miscarriage (Hertz-Picciotto et al., 1998; Ogasawara *et al.*, 2000). An abnormal karyotype of the conceptus was also detected in $>50\%$ of cases with ≤ 4 recurrent miscarriages, but less in cases with ≥ 5 miscarriages (Ogasawara *et al.*, 2000; Christiansen *et al.*, 2002). There are very few cases with an abnormal embryonal karyotype in patients with ≥ 10 miscarriages (Ogasawara *et al.*, 2000). Thus, the rate decreases with the number of abortions. We speculate that in $\sim 18\%$ of recurrent aborters the cause is embryonal chromosome abnormalities. The present study provided the possibility that elevated exposure to BPA might play a role in these cases.

Hyperprolactinaemia, hypothyroidism, elevated NK cell activity and the presence of autoantibodies are well known to be associated with recurrent miscarriages (Aoki *et al.*, 1995; Hirahara *et al.*, 1998; Ogasawara *et al.*, 1999). While BPA is reported to stimulate prolactin release, our results do not provide any support for an action in this way (Steinmetz *et al.*, 1997). Exposure to BPA does not influence prolactin production, thyroid function, NK cell activity and aPLs in the human body, in contrast to the case with animals, and the only link here was shown with ANAs. The frequency of ANAs is significantly elevated in recurrent miscarriage cases, but ANA itself does not predict subsequent miscarriage (Ogasawara *et al.*, 1996a). This is the first evidence of a link between BPA and ANA production, to our knowledge. Some drugs have been shown to exacerbate idiopathic systemic lupus erythematosus, including estrogen-containing oral contraceptives, and BPA, which has estrogen activity, could induce autoimmunity. In fact, Tian *et al.* (2003) demonstrated that interleukin-4 production was increased both *in vitro* and *in vivo* by treatment with BPA, suggesting that it might contribute to autoimmune disease.

This was a preliminary study and the sample size was relatively small. Preimplantation diagnosis might be introduced to reduce the miscarriages caused by abnormal embryonal karyotype, although in many countries, including Japan, this is not presently permitted for ethical reasons (Wilding *et al.*, 2004). However, the results suggest that further analysis of the effects of BPA on human reproduction is definitely warranted.

Acknowledgements

Supported by the Ministry of Health, Labour and Welfare, Japan.

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Submitted on September 30, 2004; resubmitted on February 22, 2005; accepted on March 3, 2005