Human Reproduction Vol.17, No.11 pp. 2839–2841, 2002

Determination of bisphenol A concentrations in human biological fluids reveals significant early prenatal exposure

Yumiko Ikezuki[1], Osamu Tsutsumi[1,2,3], Yasushi Takai[1], Yoshimasa Kamei[1] and Yuji Taketani[1]

[1]Department of Obstetrics and Gynecology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655 and [2]CREST, Japan Science and Technology, 4-1-8 Honcho, Kawaguchi, Saitama 332-0012, Japan

[3]To whom correspondence should be addressed at: Department of Obstetrics and Gynecology, Faculty of Medicine, University of Tokyo, 7-3-1 Hongo, Bunkyo-ku, Tokyo 113-8655, Japan. E-mail: osamut-tky@umin.ac.jp

BACKGROUND: There is broad human exposure to bisphenol A (BPA), an estrogenic endocrine-disrupting chemical widely used for the production of plastic products. BPA is reported to affect preimplantation embryos or fetuses and alter their postnatal development at doses typically found in the environment. We measured contamination of BPA in various kinds of human biological fluids by a novel enzyme-linked immunosorbent assay. METHODS: Blood samples were obtained from healthy premenopausal women, women with early and full-term pregnancy, and umbilical cord at full-term delivery. Ovarian follicular fluids obtained during IVF procedures and amniotic fluids obtained at mid-term and full-term pregnancy were also subject to BPA measurements. RESULTS: BPA was present in serum and follicular fluid at ~1–2 ng/ml, as well as in fetal serum and full-term amniotic fluid, confirming passage through the placenta. Surprisingly, an ~5-fold higher concentration, 8.3 ± 8.7 ng/ml, was revealed in amniotic fluid at 15–18 weeks gestation, compared with other fluids. CONCLUSION: These results suggest accumulation of BPA in early fetuses and significant exposure during the prenatal period, which must be considered in evaluating the potential for human exposure to endocrine-disrupting chemicals.

Key words: amniotic fluid/bisphenol A/endocrine disruptor/fetus/pregnancy

Introduction

Bisphenol A (BPA), an estrogenic endocrine-disrupting chemical, is produced at a value of ~1.7 billion kg per annum worldwide and used in the production of polycarbonate plastics and epoxy resins, which are used in dentistry, food packaging, and as lacquers to coat food cans, bottletops and water pipes. Thus, there is broad human exposure to BPA, which can act at the very low doses detected in the environment (Sheehan, 2000). BPA administered to pregnant mice is transferred to fetuses and alters postnatal development and sexual maturity at doses typically found in the environment (2.4 μg/kg) (Howdeshell *et al*., 1999). We also reported that BPA not only affects early embryonic development at a low, environmentally relevant level (0.23 ng/ml) (Takai *et al*., 2000), but also exerts late effects on postnatal development in mice (Takai *et al*., 2001). Moreover, many other studies have detected low dose effects of BPA in mice (Nagel *et al*., 1997; vom Saal *et al*., 1998; Gupta, 2000; Markey *et al*., 2001), rats (Steinmetz *et al*., 1997; Ramos *et al*., 2001), fish (Lindholst *et al*., 2000; Metcalfe *et al*., 2001), snails (Oehlmann *et al*., 2000), and frogs (Kloas *et al*., 1999).

Recently we measured serum BPA concentrations by a novel enzyme-linked immunosorbent assay (Kodaira *et al*., 2000) and detected BPA in all human sera (Takeuchi and Tsutsumi, 2002). Thus, it is of great importance to determine the precise levels of human and fetal contamination with BPA.

Materials and methods

We used a novel enzyme-linked immunosorbent assay (ELISA) for BPA (Kodaira *et al*., 2000; Takeuchi and Tsutsumi, 2002), which was recently developed by Otsuka Assay Laboratories and Yanaihara Institute Inc., to determine contamination in human biological fluids, obtained with informed consent. Briefly, the assay range was 0.5–5000 ng/ml of BPA. The ranges of the intra- and inter-assay coefficients of variation were 3.6–10.8 and 6.3–8.4% respectively. No cross-reactivity was observed toward other chemicals except for bis-(hydroxyphenyl)butane, bis-(hydroxyphenyl)ethane and bis-(hydroxymethylphenyl)propane (Kodaira *et al*., 2000).

Blood samples were obtained from 30 healthy premenopausal women (non-pregnant) and 37 women with early pregnancy. Thirty-seven maternal (late pregnancy) and 32 umbilical cord blood samples were also obtained at full-term delivery. Thirty-six ovarian follicular fluids were aspirated during IVF procedures. Thirty-two and 38 amniotic fluids were obtained by amniocentesis at 15–18 weeks gestation (early pregnancy) and by amniotomy at full-term Caesarean section (late pregnancy) respectively. No chromosomal abnormalities or major anomalies were found in these fetuses.

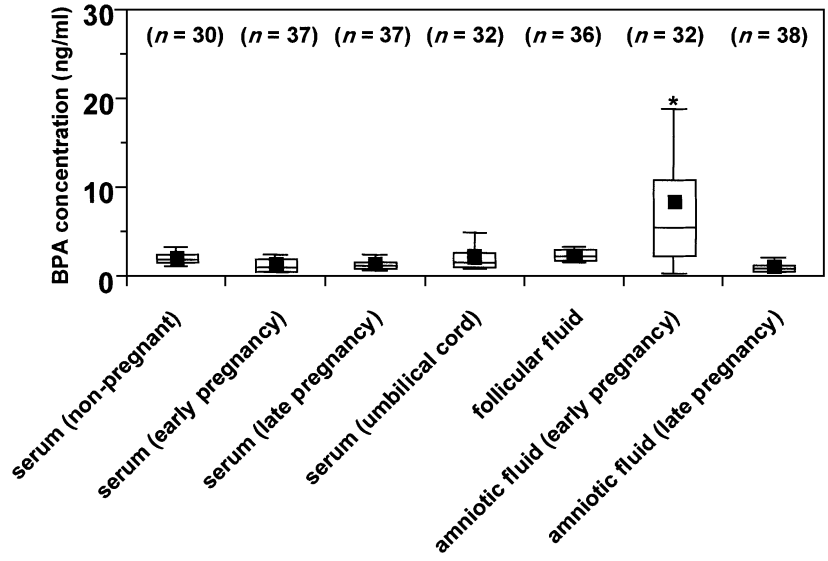

Figure 1. Bisphenol A (BPA) concentrations in human biological fluids. Data are presented as box and whisker plots, where boxes encompass values between the 25th and 75th percentiles, horizontal lines represent median values, and 'whiskers' give the 95% range of the values. Plots (■) represent mean values. Number of samples appears in parentheses. *$P < 0.0001$ compared with other biological fluids.

Statistical analyses among the groups were performed by Kruskal–Wallis test. $P < 0.05$ was chosen to indicate a statistically significant difference.

Results and discussion

As shown in Figure 1, BPA was present in serum and follicular fluid at 2.0 ± 0.8 (mean ± SD; non-pregnant), 1.5 ± 1.2 (early pregnancy), 1.4 ± 0.9 (late pregnancy) and 2.4 ± 0.8 ng/ml (follicular fluid), as well as in fetal serum (2.2 ± 1.8 ng/ml) and amniotic fluid, confirming passage through the placenta. There was no significant correlation between the maternal and fetal serum concentrations (data not shown), suggesting that BPA may be partly metabolized in the fetus. There was approximately a 5-fold difference in BPA concentrations between amniotic fluid obtained at 15–18 weeks gestation (8.3 ± 8.9 ng/ml) and other fluids ($P < 0.0001$). However, amniotic fluid levels decreased significantly at term (1.1 ± 1.0 ng/ml) and no longer differed from the levels of other fluids.

To verify the results of our ELISA for BPA in the amniotic fluid samples, we compared BPA values obtained by our ELISA with those by the conventional reverse-phase high-performance liquid chromatography (HPLC) analysis. The results showed a significant linear correlation between the two procedures ($R = 0.93$, $P < 0.0001$, data not shown). The origin of amniotic fluid in the first half of pregnancy presumably is the transudation of fluid from maternal plasma across the membranes that cover the placenta and the cord, because in composition the fluid is almost identical to a transudate of plasma. In the second half of pregnancy, there is a progressive admixture of fetal urine. From this point of view, in the first half of pregnancy BPA is transferred from maternal plasma to amniotic fluid and accumulated in the uterine cavity because of low metabolic clearance of BPA. Accordingly, a fetus is exposed to a large amount of BPA. Although its metabolism in the human body is largely unknown, BPA may be glucuronidated by a liver enzyme, uridine diphosphonate glucuronosyl transferase (UGT) (Yokota *et al*., 1999). Significantly higher levels of BPA in the mid-term amniotic fluid indicate accumulation and subsequent significant exposure, and may be explained by immature liver function including UGT activity. In the second half of pregnancy, a fetus can swallow a large amount of amniotic fluid and conjugate BPA concomitantly with the maturation of liver function. Because BPA presumably returns via placenta from fetus to mother or is diluted by fetal urine, BPA levels in amniotic fluid may decrease at term. Indeed, the HPLC analysis revealed that the percentage of glucuronidated BPA in the mid-term amniotic fluid (34 ± 9%) was less than half of that in other human biological fluids (>90%) (our unpublished observation) and in rat urine (57–84%) obtained after oral administration of BPA (Pottenger *et al*., 2000). Since it was reported that BPA glucuronidate did not show appreciable efficacy in test systems for activation of in-vitro estrogen receptors α and β (Snyder *et al*., 2000), human mid-term amniotic fluid contains not only significantly elevated total (intact plus metabolized) BPA, but also significantly elevated biologically active molecules of BPA. There is much to be elucidated about the involvement of early BPA exposure in the recent phenomena in humans, such as increased genital abnormalities in boys (Paulozzi *et al*., 1997), earlier sexual maturation in girls (Herman-Giddens *et al*., 1997), decreased sperm count in men (Carlsen *et al*., 1992), and increased breast cancer in women (Brewster and Helzlsouer, 2001).

Acknowledgments

We are grateful to Dr Yasuaki Usuki at Otsuka Assay Laboratories for help in measuring BPA concentration. We thank Drs Yutaka Osuga and Akihiko Suenaga at Tokyo University Hospital, Haruo Masuda at Tokyo Metropolitan Bokuto General Hospital, Seiichiro Obata at Hamada Hospital, Hisaya Ikeshita at Ikeshita Ladies and Children's Hospital for help in obtaining samples. We also thank the reviewers of the Journal for helpful comments which were partly incorporated into our manuscript. This study was supported in part

by grants-in-aid from the Ministry of Education, Culture, Sports, Science and Technology, from the Ministry of Health, Labour and Welfare, and from the National Institute for Environmental Studies, Japan.

References

Brewster, A. and Helzlsouer, K. (2001) Breast cancer epidemiology, prevention, and early detection. *Curr. Opin. Oncol.*, **13**, 420–425.

Carlsen, E., Giwercman, A., Keiding, N. and Skakkebaek, N.E. (1992) Evidence for decreasing quality of semen during past 50 years. *Br. Med. J.*, **305**, 609–613.

Gupta, C. (2000) Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals. *Proc. Soc. Exp. Biol. Med.*, **224**, 61–68.

Herman-Giddens, M.E., Slora, E.J., Wasserman, R.C., Bourdony, C.J., Bhapkar, M.V., Koch, G.G. and Hasemeier, C.M. (1997) Secondary sexual characteristics and menses in young girls seen in office practice: a study from the Pediatric Research in Office Settings network. *Pediatrics*, **99**, 505–512.

Howdeshell, K.L., Hotchkiss, A.K., Thayer, K.A., Vandenbergh, J.G. and vom Saal, F.S. (1999) Exposure to bisphenol A advances puberty. *Nature*, **401**, 763–764.

Kloas, W., Lutz, I. and Einspanier, R. (1999) Amphibians as a model to study endocrine disruptors: II. Estrogenic activity of environmental chemicals *in vitro* and *in vivo*. *Sci. Total Environ.*, **225**, 59–68.

Kodaira, T., Kato, I., Li, J., Mochizuki, T., Hoshino, M., Usuki, Y., Oguri, H. and Yanaihara, N. (2000) A novel ELISA for the measurement of immunoreactive bisphenol A. *Biomed. Res.*, **21**, 117–121.

Lindholst, C., Pedersen, K.L. and Pedersen, S.N. (2000) Estrogenic response of bisphenol A in rainbow trout (Oncorhynchus mykiss). *Aquatic Toxicol.*, **48**, 87–94.

Markey, C.M., Luque, E.H., Munoz De Toro, M., Sonnenschein, C. and Soto, A.M. (2001) *In utero* exposure to bisphenol A alters the development and tissue organization of the mouse mammary gland. *Biol. Reprod.*, **65**, 1215–1223.

Metcalfe, C.D., Metcalfe, T.L., Kiparissis, Y., Koenig, B.G., Khan, C., Hughes, R.J., Croley, T.R., March, R.E. and Potter, T. (2001) Estrogenic potency of chemicals detected in sewage treatment plant effluents as determined by *in vivo* assays with Japanese medaka (Oryzias latipes). *Environ. Toxicol. Chem.*, **20**, 297–308.

Nagel, S.C., vom Saal, F.S., Thayer, K.A., Dhar, M.G., Boechler, M. and Welshons, W.V. (1997) Relative binding affinity-serum modified access (RBA-SMA) assay predicts the relative *in vivo* bioactivity of the xenoestrogens bisphenol A and octylphenol. *Environ. Health Perspect.*, **105**, 70–76.

Oehlmann, J., Schulte-Oehlmann, U., Tillmann, M. and Markert, B. (2000) Effects of endocrine disruptors on prosobranch snails (Mollusca: Gastropoda) in the laboratory. Part I: Bisphenol A and octylphenol as xeno-estrogens. *Ecotoxicology*, **9**, 383–397.

Paulozzi, L.J., Erickson, J.D. and Jackson, R.J. (1997) Hypospadias trends in two US surveillance systems. *Pediatrics*, **100**, 831–834.

Pottenger, L.H., Domoradzki, J.Y., Markham, D.A., Hansen, S.C., Cagen, S.Z. and Waechter, J.M., Jr (2000) The relative bioavailability and metabolism of bisphenol A in rats is dependent upon the route of administration. *Toxicol. Sci.*, **54**, 3–18.

Ramos, J.G., Varayoud, J., Sonnenschein, C., Soto, A.M., Munoz De Toro, M. and Luque, E.H. (2001) Prenatal exposure to low doses of bisphenol A alters the periductal stroma and glandular cell function in the rat ventral prostate. *Biol. Reprod.*, **65**, 1271–1277.

Sheehan, D.M. (2000) Activity of environmentally relevant low doses of endocrine disruptors and the bisphenol A controversy: initial results confirmed. *Proc. Soc. Exp. Biol. Med.*, **224**, 57–60.

Snyder, R.W., Maness, S.C., Gaido, K.W., Welsch, F., Sumner, S.C. and Fennell, T.R. (2000) Metabolism and disposition of bisphenol A in female rats. *Toxicol. Appl. Pharmacol.*, **168**, 225–234.

Steinmetz, R., Brown, N.G., Allen, D.L., Bigsby, R.M. and Ben-Jonathan, N. (1997) The environmental estrogen bisphenol A stimulates prolactin release *in vitro* and *in vivo*. *Endocrinology*, **138**, 1780–1786.

Takai, Y., Tsutsumi, O., Ikezuki, Y., Hiroi, H., Osuga, Y., Momoeda, M., Yano, T. and Taketani, Y. (2000) Estrogen receptor-mediated effects of a xenoestrogen, bisphenol A, on preimplantation mouse embryos. *Biochem. Biophys. Res. Commun.*, **270**, 918–921.

Takai, Y., Tsutsumi, O., Ikezuki, Y., Kamei, Y., Osuga, Y., Yano, T. and Taketan, Y. (2001) Preimplantation exposure to bisphenol A advances postnatal development. *Reprod. Toxicol.*, **15**, 71–74.

Takeuchi, T. and Tsutsumi, O. (2002) Serum bisphenol A concentrations showed gender differences, possibly linked to androgen levels. *Biochem. Biophys. Res. Commun.*, **291**, 76–78.

vom Saal, F.S., Cooke, P.S., Buchanan, D.L., Palanza, P., Thayer, K.A., Nagel, S.C., Parmigiani, S. and Welshons, W.V. (1998) A physiologically based approach to the study of bisphenol A and other estrogenic chemicals on the size of reproductive organs, daily sperm production, and behavior. *Toxicol. Ind. Health*, **14**, 239–260.

Yokota, H., Iwano, H., Endo, M., Kobayashi, T., Inoue, H., Ikushiro, S. and Yuasa, A. (1999) Glucuronidation of the environmental oestrogen bisphenol A by an isoform of UDP-glucuronosyltransferase, UGT2B1, in the rat liver. *Biochem. J.*, **340**, 405–409.

Submitted on March 28, 2002; accepted on July 23, 2002