Bisphenol-A Administration during Pregnancy Results in Fetal Exposure in Mice and Monkeys

Kaoru Uchida,[a, b, c] Atsuko Suzuki,[a, d] Yoshio Kobayashi,[e] David Lee Buchanan,[a, c] Tomomi Sato,[d] Hajime Watanabe,[a, c] Yoshinao Katsu,[a, c] Juri Suzuki,[f] Kazuo Asaoka,[f] Chisato Mori,[c, g] Koji Arizono,[c, h] and Taisen Iguchi*[, a, b, c, d]

[a]Center for Integrative Bioscience, Okazaki National Research Institutes, Higashiyama 5–1, Myodaiji, Okazaki 444–8585, Japan, [b]Department of Molecular Biomechanics, The Graduate University for Advanced Studies, Higashiyama 5–1, Myodaiji, Okazaki 444–8585, Japan, [c]CREST, Japan Science and Technology Corporation, 4–1–8 Honmachi, Kawaguchi 332–0012, Japan, [d]Graduate School of Integrated Science, Yokohama City University, 22–2 Seto, Kanazawa-ku, Yokohama 236–0027, Japan, [e]Technical Laboratory, Analysis Center Corporation, 1–12–2 Higashimukoujima, Sumida-ku, Tokyo 131–0032, Japan, [f]Primate Research Institute, Kyoto University, Inuyama, Aichi 484–8506, Japan, [g]Department of Anatomy and Cell Biology, School of Medicine, Chiba University, 1–8–1 Inohana, Chuo-ku, Chiba 260–8670, Japan, and [h]Faculty of Environmental and Symbiotic Sciences, Prefectural University of Kumamoto, Tsukide 3–1–100, Kumamoto 862–8502, Japan

(Received August 14, 2002; Accepted September 7, 2002; Published online September 18, 2002)

Placental transfer of bisphenol-A (BPA) was studied in mice and Japanese monkeys (*Macaca fuscata*). BPA was found in maternal and fetal sera, liver, brain, uteri, testes and placenta as early as 30 min after a single subcutaneous (s.c.) injection to 17 days of pregnancy in mice. BPA was also found in fetal liver, kidney, and brain of Japanese monkeys 1 hr after a single s.c. injection to 150 days of pregnancy. These results clearly indicate that the maternal placental barrier can not protect the fetus from the consequences of BPA exposure in these species.

Key words —— bisphenol-A, pregnancy, fetus, mouse, monkey

*To whom correspondence should be addressed: Center for Integrative Bioscience, Okazaki National Research Institutes, Higashiyama 5–1, Myodaiji, Okazaki 444–8585, Japan. Tel.: +81-564-59-5235; Fax: +81-564-59-5236; E-mail: taisen@nibb.ac.jp

INTRODUCTION

Bisphenol A (BPA) is a compound used for manufacture of the plastic polycarbonate. Estrogenic activity of BPA has been reported for over 50 years.[1)] Krishnan *et al*.[2)] reported BPA is released from autoclaved polycarbonate flasks and estrogenic activity of BPA is mediated via the estrogen receptor (ER). In serum from adult men, BPA showed a higher relative binding affinity than in a serum-free medium,[3)] showing that the estrogenic activity of BPA is more active *in vivo* than *in vitro*. Steinmetz *et al*.[4)] indicated that BPA induced the molecular and morphological alterations in uterus and vagina of adult rats. Results of some studies have shown that estrogenic chemicals including BPA and a synthetic estrogen, diethylstilbestrol (DES) can act at very low doses in the range of human and wildlife environmental exposures.[5,6)] Howdeshell *et al*.[7)] demonstrated that a very low dose of BPA during pregnancy induces early onset of first estrus. ER binding affinity for BPA has been reported to have 1/10000 potency of 17β-estradiol (E_2).[8)]

Perinatal exposure to natural and synthetic estrogens induces irreversible changes in estrogen target tissues.[9,10)] We recently found that low dose of BPA *in utero* accelerated vaginal opening in mice,[11)] and a large dose of BPA given neonatally induced ovary-independent vaginal epithelial changes.[12)] On the other hand, BPA was found in canned drinks in Japan,[13)] therefore, food contamination of BPA is possible. Developing fetus is more sensitive to estrogenic chemicals than adults in induction of various abnormalities.[9,10)] In this study, therefore, we investigated whether or not BPA cross the placenta and reach fetal tissues using mice and Japanese monkeys.

MATERIALS AND METHODS

Mice of ICR/Jcl strain kept under 12 hr light/12 hr dark at 23–25°C were given a commercial diet (CE-2, CLEA, Tokyo, Japan) and tap water *ad libitum*. Japanese monkeys (*Macaca fuscata*) kept in a room with a temperature rage between 10 to 25°C and a lighting schedule of 12 hr light/12 hr dark (light on at 6:00). All monkeys were fed every day with 160–180 g commercial moneky-chow (Oriental Yeast Co. Ltd., Tokyo, Japan) supplemented with sweet potatoes (*ca*. 100 g) three times a week. Water was given *ad libitum*. All procedures were

carried out according to the NIH Guide for the Care and Use of Laboratory Animals and approved by committees of experimental animals in National Institute for Basic Biology for mice and Primate Research Institute, Kyoto University for monkeys.

Bisphenol-A (BPA, GL Science Inc., Japan) was dissolved in sesame oil. Unless otherwise mentioned, all materials were obtained from Wako Pure Chemical Industries, Osaka, Japan. Female and male mice were cohabited and the appearance of a vaginal plug was considered the beginning of pregnancy and gestational day (GD) 0.

On GD 17 of pregnancy, ICR mice were given a single s.c. injection of 100 mg BPA/kg body weight (BW) and sacrificed at 0.5, 1, 2, 3, 6, 12 and 24 hr later. In each time point, more than 3 pregnant mice were used. Six pregnant mice were used for non-treated controls. Fetal mouse uteri and testes were pooled, therefore, only one measurement in each time point was conducted. On GD 150 of pregnancy, 2 Japanese monkeys were given a single s.c. injection of 50 mg BPA/kg BW and the fetus was dissected out by Caesarean section 1 hr after the injection. The fetuses dissected from two pregnant monkeys by Caesarean section under the 5 mg/kg BW ketamine hydrochloride and 1 mg/kg BW xylazine hydrochloride anesthesia on GD 150 were used as non-treated controls.

Maternal and fetal sera, brain, liver, kidney, fetal testes, fetal uteri, and placenta were collected in mice, and maternal and fetal sera, umbilical cord, heart, intestine, liver, spleen, kidney, thymus, muscle, cerebrum, pons and cerebellum were collected in monkeys. These samples were frozen using liquid nitrogen and stored at –80°C until BPA extraction. BPA concentrations in water, sweet potatoes, and commercial diet for monkeys were also measured. Concentrations of BPA in various organs, blood and food were analyzed by gas chromatography-mass spectrometry (GC-MS) as described previously.[14,15]

The recovery correction was made for BPA-d16 based on the recovery of BPA. The relative standard deviation of the analytical value was 1.6% for BPA (n = 3). The analyses of the extracts spiked with 100 ng of BPA showed the recovery of 102%. In cases where detected amounts of the target compounds in samples were more than twice of the amount in the procedural blank analyzed together, the detection was regarded as significant. Normally the amount of target compounds detected in the procedural blanks was ~5 ng for BPA.

RESULTS AND DISCUSSION

In utero exposure to BPA (10 mg/kg BW) reduced ovulatory activity in mice at 40 days of age. BPA (10 and 100 mg/kg BW) exposed females gave birth when mated with untreated males, and the number of pups and sex ratio were not different from those of controls.[12] Howdeshell *et al.*[7] demonstrated that exposure to BPA (2.4 μg/kg BW) *in utero* advances puberty and increases body weight in female offspring. Gupta[16] also showed that mice fed with BPA (50 mg/kg/day) and arochlor 1016 (50 μg/kg/day) had enhanced anogenital distance, increased prostate size, decreased epididymal weight and increased androgen receptor binding activity of the prostate. We recently found that BPA (20 μg/kg BW) from days 11–17 of pregnancy accelerated vaginal opening but not body weight gain in mice.[11] Neonatal exposure to a high dose of BPA (150 μg per pup) but not 15 μg BPA, induced ovary-independent vaginal changes, uterine epithelial strarification and polyovular follicles, and infertility lacking corpora lutea.[12] Several reports indicated that *in utero* exposure to BPA results in various effects on male and female mice as above and these results suggest that placental transfer of BPA into fetus. In fact, oral administration of BPA transferred from the maternal rat to the fetus.[17] In mice, BPA was found in maternal and fetal sera, liver, brain, placenta, and fetal uteri and testes as early as 30 min after injection (Fig. 1). BPA concentrations in serum and liver of fetus were higher than those of mothers. High BPA concentration in the fetal uterus may be correlated to the presence of ER in fetal uterus.[10] BPA can be found in canned drinks up to 213 ppb[13] and in river water in Japan as described.[18] BPA and other chemicals, such as dioxins, PCBs, DDTs, BHC, cadmium, lead, and nonylphenol were found in the human umbilical cord.[19,20] Thus, BPA levels were also investigated in primates, Japanese monkey. Japanese monkeys were injected with 50 mg BPA/kg BW on day 150 of pregnancy, and fetuses were collected 1 hr later. BPA was found in all organs investigated including fetal liver, kidney, brain and umbilical cord in BPA-treated monkey and even non-treated controls (0.02–22.8 μg/g) (Table 1). These values increased to 1.7–72.5 μg/g in BPA-injected monkeys 1 hr after the injection. These results indicate that the placental barrier can not protect the fetus from the consequences of direct BPA exposure. Organ specific accumulation is probable. BPA was found in commercial diet for monkeys (0.04–0.21 μg/g) and

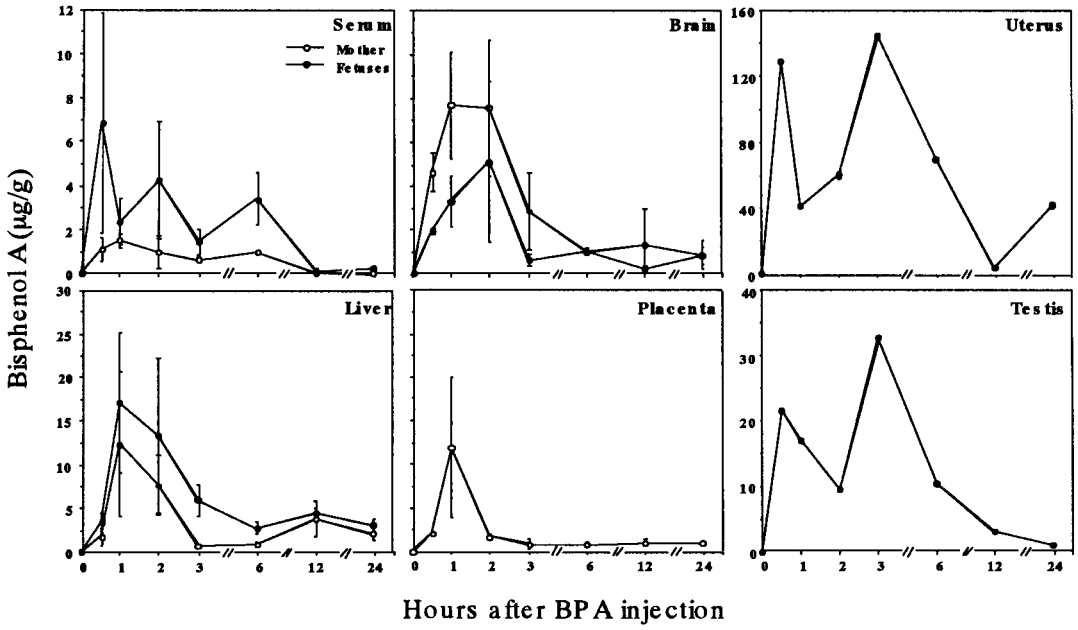

Fig. 1. Bisphenol A Levels in Mouse

On GD 17 of pregnancy, ICR mice were given a single s.c. injection of 100 mg BPA/kg BW and sacrificed at 0.5, 1, 2, 3, 6, 12 and 24 hr later. Concentrations of Bisphenol A in tissues were measured. Data represent the mean and standard error (n = 3–5).

Table 1. Bisphenol-A Concentrations in Mother Serum and Fetal Organs 1 hr after a Single Injection of 50 mg/kg BPA in Japanese Monkeys

Organs	Non-treated (μg/g)	BPA-injected (μg/g)
Mother serum	0.02	6.10
Fetal serum	0.04	1.70
Umbilical cord	0.25	5.80
Heart	3.70	36.90
Intestine	5.40	37.00
Liver	7.60	65.00
Spleen	9.70	15.60
Kidney	12.40	37.50
Thymus	17.00	30.30
Muscle	3.80	43.00
Cerebrum	14.50	52.50
Pons	22.80	50.30
Cerebellum	10.00	72.50

Values indicate mean of 2 samples. One male and one female fetuses in non-treated group and two male fetuses in BPA-treated group.

even potatoes (0.01 μg/g). The concentration of BPA in food may contribute the BPA found in non-treated monkey fetal organs. In non-treated rat, BPA was detected in sera of both mother and fetus, and in breast milk and liver.[21] Cytochrome P450s work to decrease estrogens,[22,23] and glucuronosyltransferase (GT) catalizes the glucuronide formation of BPA.[24] Since the fetus does not express GT and BPA passes through the placenta, the fetus is indefensible against any effects by this exogenous estrogen. While the newborn rat poorly expresses GT, it is also possible that perinatal exposure to BPA could affect reproductive organ development. In conclusion, BPA can be found even in the commercial diet and BPA easily pass through the placenta and reaches into fetal organs. Thus, further studies are needed to determine BPA contamination sites in experimental animals and humans in order to devoid of BPA exposure.

Acknowledgements This work was partly supported by a Grant-in-Aid for Scientific Research on Priority Areas (A) from the Ministry of Education, Science and Culture of Japan, Special Coordination Funds of Science and Technology Agency of Japanese Government, and the Health Sciences Research Grant from the Ministry of Health, Labour and Welfare, Japan. The present work has been supported by the Cooperation Research Program of Primate Institute, Kyoto University.

REFERENCES

1) Dodds, E. C. and Lawson, W. (1936) Synthetic estrogenic agents without the phenanthrene nucleus. *Nature* (London), **137**, 996–997.

2) Krishnan, A. V., Stathis, P., Permuth, S. F., Tokes, L. and Feldman, D. (1993) Bisphenol-A: An estrogenic substance is released from polycarbonate flasks during autoclaving. *Endocrinology*, **132**, 2279–2286.
3) 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.
4) Steinmetz, R., Mitchner, N. A., Grant, A., Allen, D. L., Bigsby, R. M. and Ben-Jonathan, N. (1998) The xenoestrogen bisphenol A induces growth, differentiation, and c-fos gene expression in the female reproductive tract. *Endocrinology*, **139**, 2741–2747.
5) vom Saal, F. S., Timms, B. G., Montano, M. M., Palanza, P., Thayer, K. A., Nagel, S. C., Dhar, M. D., Ganjam, V. K. and Parmigiani, S. (1997) Prostate enlargement in mice due to fetal exposure to low doses of estradiol or diethylstilbestrol and opposite effects at high doses. *Proc. Natl. Acad. Sci. U.S.A.*, **94**, 2056–2061.
6) Sheehan, D. M., Willingham, E., Gaylor, D., Bergeron, J. M. and Crews, D. (1999) No threshold dose for oestradiol-induced sex reversal of turtle embryos: how little is too much? *Environ. Health Perspect.*, **107**, 155–159.
7) Howdeshell, K. L., Hotchkiss, A. K., Thayer, K. A. A., Vandenbergh, J. G. and vom Saal, F. S. (1999) Exposure to bisphenol A advances puberty. *Nature* (London), **401**, 763–764.
8) Kuiper, G. G., Lemmen, J. G., Carlsson, B., Corton, J. C., Safe, S. H., van der Saag, P. T., van der Burg, B. and Gustafsson, J. A. (1998) Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology*, **139**, 4252–4263.
9) Takasugi, N. (1976) Cytological basis for permanent vaginal changes in mice treated neonatally with steroid hormones. *Int. Rev. Cytol.*, **44**, 193–224.
10) Iguchi, T. (1992) Cellular effects of early exposure to sex hormones and antihormones. *Int. Rev. Cytol.*, **139**, 1–57.
11) Honma, S., Suzuki, A., Buchanan, D. L., Katsu, Y., Watanabe, H. and Iguchi, T. (2002) Low dose effect of in utero exposure to bisphenol A and diethylstilbestrol on female mouse reproduction. *Reprod. Toxicol.*, **16**, 117–122.
12) Suzuki, A., Sugihara, A., Uchida, K., Sato, T., Ohta, Y., Katsu, Y., Watanabe, H. and Iguchi, T. (2002) Developmental effects of perinatal exposure to bisphenol-A and diethylstilbestrol on reproductive organs in female mice. *Reprod. Toxicol.*, **16**, 107–116.
13) Kawamura, Y., Sano, H. and Yamada, T. (1999) Migration of bisphenol A from can coatings to drinks. *J. Food Hyg. Soc. Japan*, **40**, 158–165.
14) Isobe, T., Nishiyama, H., Nakashima, A. and Takada, H. (2001) Distribution and behavior of nonylphenol, octylphenol, and nonylphenol monoethoxylate in Tokyo metropolitan area — their association with aquatic particles and sedimentary distributions. *Environ. Sci. Technol.*, **35**, 1041–1049.
15) Nakada, N., Isobe, T., Nishiyama, H., Okuda, K., Tsutsumi, S., Yamada, J., Kumata, H. and Takada, H. (1999) Broad-spectrum analysis of endocrine disrupters in environmental samples. *Bunseki Kagaku*, **48**, 535–547.
16) Gupta, C. (2000) Reproductive malformation of the male offspring following maternal exposure to estrogenic chemicals. *Proc. Soc. Exp. Biol. Med.*, **224**, 61–68.
17) Miyakoda, H., Tabata, M., Onodera, S. and Takeda, K. (1999) Passage of bisphenol A into the fetus of the pregnant rat. *J. Health Sci.*, **45**, 318–323.
18) Iguchi, T., Watanabe, H. and Katsu, Y. (2001) Developmental effects of estrogenic agents on mice, fish and frogs: a mini review. *Horm. Behav.*, **40**, 248–251.
19) Takada, H., Isobe, T., Nakata, N., Nishiyama, H., Iguchi, T., Irie, H. and Mori, C. (1998) Detection of bisphenol A and nonylphenols in umbilical cords. In *Abstract of 1st Ann. Meet. Japan Soc. Endocr. Disrupt. Res.*, Kyoto, p. 34.
20) Mori, C. (2001) Possible effects of endocrine disruptors on male reproductive function: a mini review. *Acta Anat. Nippon*, **76**, 361–368.
21) Yoshida, M., Katsuda, S.-I., Watanabe, G., Taya, K. and Maekawa, A. (2001) Effects of in utero and lactational exposure to low-doses of bisphenol A on the reproductive system in female rats. In *Proc. 28th Ann. Soc. Toxicol. Japan*, Abstract 012–016.
22) Robl, J. M., Thomford, P. J., Wu, M. C. and Dziuk, P. J. (1985) Effects of estrogen, testosterone, and phenobarbital on uterine weight and liver microsomal enzymes in prepubertal mice. *Pediatr. Pharmacol.* (New York), **5**, 157–164.
23) Shah, H. C. and McLachlan, J. A. (1976) The fate of diethylstilbestrol in the pregnant mouse. *J. Pharmacol. Exp. Ther.*, **197**, 687–696.
24) 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.