Differences in Serum Bisphenol A Concentrations in Premenopausal Normal Women and Women with Endometrial Hyperplasia

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Abstract. Exposure to endocrine disrupting chemicals (EDCs) has been raised in relation to its potential for adverse health outcomes. Bisphenol A (BPA) is an estrogenic EDC widely found in plastic products. We determined BPA concentrations in premenopausal women by an enzyme-linked immunosorbent assay and evaluated possible linkage between its contamination levels and endometrial hyperplasia, an estrogen-related disorder of the uterus. It has been implied that higher levels of BPA, which binds to estrogen receptor and plays estrogenic roles may, enhance endometrial hyperplasia. Serum BPA was detectable in all subjects and its concentrations in healthy controls with normal endometrium were 2.5 ± 1.5 ng/ml (mean ± SD). BPA levels in patients with simple endometrial hyperplasia with benign nature were 2.9 ± 2.0 ng/ml and were not significantly different from the controls. Unexpectedly, BPA levels in patients with complex endometrial hyperplasia with malignant potential were 1.4 ± 0.4 ng/ml and significantly lower compared to both control and simple endometrial hyperplasia groups. In addition, we measured the serum BPA levels in postmenopausal endometrial cancer patient (1.4 ± 0.5 ng/ml), which were also significantly lower than control and simple endometrial hyperplasia groups. These findings suggest the presence of associations between BPA exposure and complex endometrial hyperplasia and endometrial cancer. The mode of action of BPA may be more complex than expected and the contradictory results may serve as a clue to addressing the mechanisms of linkage between occurrence of estrogen-dependent diseases and endocrine disruption.

Key words: Bisphenol A, Simple endometrial hyperplasia, Complex endometrial hyperplasia, Endometrial carcinoma

ENDOCRINE disrupting chemicals (EDCs) have generated growing scientific concern and public debate over their potential adverse effects that may result from their exposure. Substantial evidence has been accumulated that point to their potential to alter the normal function of the endocrine system in wildlife and humans [1]. The International Programme on Chemical Safety provided an objective, global assessment of the current state-of-the-science relative to environmental endocrine disruption in humans, experimental studies, and wildlife species and defined an EDC as an exogenous substance or mixture that alters function (s) of the endocrine system and consequently causes adverse health effects in an intact organism, or its progeny, or (sub) populations [2]. Temporal increases in the incidence of certain cancers in hormonally sensitive tissues in many parts of the industrialized world [3, 4] are often cited as evidence that widespread exposure of the general population to EDCs has had adverse impact on human health. These increases cannot be adequately explained by improved diagnostic techniques but it has yet to be clarified whether these trends merely coincide with the increased use and release of EDCs into the environment. There is, therefore, a concern that EDCs may increase estrogen dependent disorders including endometrial malignancies through their estrogenic effects.
Bisphenol A (BPA), an estrogenic EDC with two unsaturated phenol rings, is widely used in the production of polycarbonate plastic and epoxy resin, which are used in dentistry, food packaging, and as lacquers to coat food cans, bottletops and water pipes [5, 6]. A significant amount of BPA was detected in liquid from canned vegetables that are exposed to high temperature during autoclaving [7] and in saliva of dental patients fitted with restorative materials [8]. Bioaccumulation of BPA occurs in water and its concentrations ranged 0.02–0.15 microg/L in river water, while they ranged 2–8.8 microg/kg-wet in the periphytons and 0.3–12 microg/kg-wet in the benthos [9]. BPA has been reported to bind estrogen receptors (ERα and ERβ) and play either estrogenic or anti-estrogenic roles in vitro [10, 11]. BPA has been shown to have several actions such as uterotrophic effects [12, 13], decreasing sperm production [14], stimulation of prolactin release [15], and promotion of cell proliferation in a breast cancer cell line [7], by animal experiments. It was reported to have a promotive effect on growth and puberty by fetal or preimplantation exposure of environmental doses of BPA in mice [16, 17]. To study human contamination of BPA, we employed enzyme-linked immunosorbent assay (ELISA) and reported that BPA was detectable not only in the serum but several biological fluids including amniotic fluids demonstrating human fetal exposure to BPA through placenta [18]. It is interesting to note there was a gender difference in serum BPA concentrations in humans [19] and more recently, determination of serum BPA concentrations in women with or without ovarian dysfunction revealed that BPA levels may vary according to the endocrinological circumstances in the subjects [20].

Since studies that clearly address exposure-outcome relationships may be the most valuable means of assessing the impact of EDCs on human health, we measured serum BPA levels in patients with endometrial hyperplasia and endometrial cancer in order to investigate the relation of BPA to these estrogen-related disorders in the uterus. Because BPA mimics estrogen action via estrogen receptors in the endometrium of the uterus it is conceivable that elevated levels of BPA may cause malignant hyperplastic endometrial disorders, which are known to be caused by persistent and prolonged exposure of estrogen. On the contrary, our results showed that BPA levels in those patients were unexpectedly low compared to normal controls.

Materials and Methods

Subjects

Patients recruited in this study came to the outpatient clinic in Hitachi General Hospital (Ibaraki, Japan) or Tokyo University Hospital. Nineteen patients were diagnosed as endometrial hyperplasia by endometrial biopsy. They were divided into two groups by their glandular complexity and crowding. Ten patients with lesser degrees of glandular complexity and crowding were designated as simple hyperplasia and 9 patients with marked glandular complexity and crowding producing a back-to-back appearance were designated as complex hyperplasia by histological specialists. Seven patients with endometrial carcinoma group were pathologically diagnosed by histological specialists. Eleven subjects whose cytology of uterine endometrium was negative served as healthy controls. Informed consent was obtained from all subjects in the present study and the study was approved by the Research and Ethics committee of the University of Tokyo.

BPA measurement

Blood samples were obtained at the time of endometrial examination and all the subjects had free access to food and water before the examination. Serum BPA levels were assayed with a competitive ELISA [21]. Briefly, after a 1 ml serum sample was washed with 10% methanol, the eluate was obtained from a solid-phase column (Oasis HLB column) with the sample in 1 ml of methanol:acetonitrile (3:1 v/v), and the solvent was evaporated under a stream of nitrogen gas. An aliquot (0.2 ml) of phosphate buffer was added to the dry residue in the test tube. Fifty µl of the sample extract and 50 µl of peroxidase-labelled BPA were placed on microtitre plates coated with a solid phase containing rabbit anti-BPA polyclonal antibody, and incubated at room temperature for 2 hours. After washing, an aliquot (0.1 ml) of orthophenylenediamine was added as a luminescence substrate and the plate was allowed to stand for 30 min. The reaction was stopped by addition of 0.1 ml 1 N H2SO4. The optical absorbance was measured at a wavelength of 490 nm by a micro-plate reader in a vertical-beam photometer. A standard curve was prepared for the analysis. The cross-reaction substances were bis (4-hydroxyphenyl) methane (0.8%), 1,1-bis (4-hydroxyphenyl) ethane (10.4%), 2,2-bis
(hydroxyphenyl) butane (40.9%), bis (hydroxymethylphenyl) propane (1.6%), and other related substances (<0.1%). The intra- and inter-assay coefficients of variance were 7.7% and 9.7%. A significant correlation (r = 0.971) was confirmed between the BPA values obtained from the HPLC analysis and ELISA.

Statistical analysis

Data are presented as means ± standard deviation (SD). The differences between each value were evaluated by analysis of variance and Student's *t*-test except for gravidity and parity which were compared by chi-square test.

Results

There were no significant differences in the age, gravidity, parity, body height, body weight and body mass index (BMI) among control healthy subjects, patients with simple endometrial hyperplasia and those with complex endometrial hyperplasia (Table 1). All 30 of these subjects were in premenopausal period. Patients with endometrial cancer were over-aged and in postmenopausal period. They were characterized with significantly lower gravidity and parity and their height and weight were also significantly lower than those of other groups. However, BMI of endometrial cancer patients in the present study was not significantly different from those of other groups.

Serum BPA was detectable in all subjects tested in the present study and its concentrations in healthy premenopausal controls with normal endometrium were 2.5 ± 1.5 ng/ml (M ± SD, n = 11) (Table 1). BPA levels in 19 patients with endometrial hyperplasia as a whole were 2.2 ± 1.6 ng/ml and were not significantly different from the controls. When they were divided into two groups of simple endometrial hyperplasia with benign nature and complex endometrial hyperplasia with malignant potential, however, there was a significant difference between simple endometrial hyperplasia patients (2.9 ± 2.0, n = 10) and complex endometrial hyperplasia patients (1.4 ± 0.4 ng/ml, n = 9). Serum BPA levels were significantly lower in complex endometrial hyperplasia patients compared to control group. In addition, we measured the serum BPA levels in postmenopausal endometrial cancer patient. BPA concentrations were 1.4 ± 0.5 ng/ml (n = 7) and were also significantly lower than those of control and simple endometrial hyperplasia groups.

![Fig. 1. Comparison of serum bisphenol A (BPA) concentrations among the study groups.](image)

*Fig. 1.* Comparison of serum bisphenol A (BPA) concentrations among the study groups. Serum BPA concentrations were significantly lower in both complex endometrial hyperplasia and endometrial cancer patients compared with those in normal women simple endometrial hyperplasia.

*P<0.05; compared with normal control group, *P<0.01; compared with simple endometrial hyperplasia groups.

<table>
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<th>Subjects</th>
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<th>parity</th>
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<th>weight</th>
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<td>2.2 ± 1.0</td>
<td>153.5 ± 3.5</td>
<td>56.0 ± 8.2</td>
<td>23.8 ± 3.5</td>
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<td>156.3 ± 5.7</td>
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<td>24.2 ± 2.4</td>
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<tr>
<td>CEH</td>
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<td>48.4 ± 3.6</td>
<td>2.9 ± 1.5</td>
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<td>157.6 ± 7.4</td>
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<tr>
<td>Endometrial cancer</td>
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<td>1.7 ± 1.5*</td>
<td>1.3 ± 1.2*</td>
<td>151.4 ± 7.5*</td>
<td>50.7 ± 5.3*</td>
<td>22.2 ± 2.9</td>
</tr>
</tbody>
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*P<0.05; compared with other groups.
Discussion

BPA contamination in humans has been demonstrated in the present study confirming previous reports showing that BPA is detectable in all human serum samples [18–20]. Differences in serum BPA concentrations between normal women and patients with complex endometrial hyperplasia are first described in the present study. To our knowledge, it is also the first report to address the possible relationship between BPA contamination and estrogen-related disorders in humans.

BPA concentrations in the premenopausal and postmenopausal women were approximately 1–2 ng/ml, which were the same levels as reported by Ikezuki et al. in premenopausal and pregnant women [18]. BPA levels reported by Takeuchi et al. [19, 20] were lower than those in the present study and Ikezuki et al. The difference may be explained by the way the serum samples were obtained. The data by Takeuchi et al. were obtained from fasting subjects, while the subjects were allowed free access to food and water in those by Ikezuki and the present study. Since BPA exposure occurs via food and water and BPA has a relatively short half-life unlike other EDCs such as dioxins, dietary condition may be a very important factor to assess BPA contamination. BPA contamination levels in the present study were the same as found in the environment such as in river water [9]. BPA contamination in both humans and environment may be significant because BPA effects in in vitro experiment were found to be less than these concentrations [17].

Endometrial hyperplasia is assumed to result from persistent, prolonged estrogenic stimulation of the endometrium [22]. The most common cause is a succession of anovulatory cycles, but hyperplasia may also result from exogenously administered or excessive endogenously-produced estrogen. Endometrial hyperplasia may occur in association with granulose cell tumors, ovarian thecomas, polycystic ovarian syndrome and adrenocortical hyperplasia. These relationships can all be interpreted in the light of the “unopposed estrogen” hypothesis [23, 24], which proposes that endometrial hyperplasia and endometrial cancer may develop as a result of the mitogenic effects of estrogens, when these are insufficiently counterbalanced by progesterone. The endometrial response to unopposed estrogen can be viewed as a spectrum of changes ranging from benign to malignant. The risk of endometrial cancer for patients with complex endometrial hyperplasia is higher than that of patients with simple endometrial hyperplasia [25].

Since BPA may mimic estrogen action in the endometrium of the uterus it is conceivable that elevated levels of BPA may cause endometrial hyperplasia. However, there was no difference between normal and simple endometrial hyperplasia groups. On the contrary, BPA levels in complex endometrial hyperplasia were significantly lower than those with normal women and patients with simple endometrial hyperplasia. The result that BPA levels in patients with endometrial cancer is also lower than those with normal women and patients with simple endometrial hyperplasia suggests us there may be some relationship between BPA concentrations and malignant change in the endometrium. It is, however, difficult to explain the mechanism in which lower BPA levels causes the changes in the endometrium. Dietary amounts of BPA ingested may not differ among these experimental groups. One possible explanation for decreased BPA levels may be metabolic capability for BPA is increased in patients with complex endometrial hyperplasia and endometrial cancer although the precise mechanism is yet unknown. Alternatively, it is plausible that BPA exerts anti-estrogenic roles in the human endometrium, and that lower BPA levels are a favorable environment to grow complex endometrial hyperplasia and endometrial cancer.

Indeed, BPA acts as an anti-estrogenic role in several cell lines in the presence of estrogen through ERα, and we have shown that 1 μM of BPA exerts antagonistic actions in the presence of 10 nM estradiol 17β [10], suggesting that the mode of action of endocrine disruptors are more complex than thought. Presence of BPA in the human endometrium [26] supports the idea that there may be possible interactions between BPA and endogenous estrogen in the local environment. Complex role of BPA in testicular steroidogenesis is also reported recently [27].

In the present study, we investigated serum BPA levels in endometrial hyperplasia and endometrial carcinoma cases. Our preliminary results demonstrated a possible linkage between BPA exposure and estrogen-dependent endometrial disorders and that the modes of actions for environmentally produced estrogen-like substances are more complex than expected. At present, the biological influences of the substance remains to be clarified. Exposure assessment must focus on vulnerable groups, in terms of both life stage and life-
style. Vulnerability of different groups in the population may be affected by lifestyle factors, genetic factors (metabolic differences that can determine sensitivity), special habits, and age. The present results offer clues to future studies and more studies are needed to clarify if and how EDCs affect human health.

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References


