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Accumulation of Bisphenol A in Hemodialysis Patients

Kazutaka Murakami[a] Atsushi Ohashi[b] Hideo Hori[b] Makoto Hibiya[b] Yumiko Shoji[a] Miyuki Kunisaki[a] Miho Akita[a] Akira Yagi[a] Kazuhiro Sugiyama[a] Sachiko Shimozato[a] Kazuhiro Ito[a] Hiroki Takahashi[a] Kazuo Takahashi[a] Kouichirou Yamamoto[a] Masami Kasugai[a] Nahoko Kawamura[a] Shigeru Nakai[a] Midori Hasegawa[a] Makoto Tomita[a] Kunihiro Nabeshima[a] Yoshiyuki Hiki[a] Satoshi Sugiyama[a]

[a]Department of Nephrology, Fujita Health University School of Medicine, and
[b]Department of Clinical Biochemistry, Fujita Health University, Toyoake, Japan

Key Words
Bisphenol A · Endocrine disrupter · Hemodialysis

Abstract
Bisphenol A [BPA, 2,2-bis(4-hydoxyphenyl)propane], an industrial chemical used in the production of polycarbonate, epoxide resin, and polyarylate, is considered to be an endocrine-disrupting chemical. BPA may be present in some hollow-fiber dialyzers used in hemodialysis. In this study, we tested the amounts of BPA eluted from various hollow fibers. Furthermore, we measured the BPA concentration in the sera of 22 renal disease predialysis patients, as well as 15 patients who were receiving hemodialysis, to see if there is BPA accumulation in these patients. The elution test of BPA showed that a much larger amount of BPA was eluted from polysulfone (PS), and polyester-polymeralloy hollow fibers. Among renal disease patients who had not undergone hemodialysis, the serum BPA concentration increased as the renal function deteriorated, showing a significant negative association. In a crossover test between PS and cellulose (Ce) dialyzers, the predialysis serum BPA concentration of PS dialyzer users decreased after changing to a Ce dialyzer, and the serum BPA increased again after switching back to PS dialyzers. In patients who were using PS dialyzers, the BPA level significantly increased after a dialysis session. However, in the Ce dialyzer users, the BPA level decreased. Since accumulation of BPA could affect the endocrine or metabolic system of the human body, it is important to perform further investigations on dialysis patients.

Introduction

The hormone-like action of some chemical substances existing in wild animals, which are called endocrine disrupters, has been indicated to be associated with reproductive dysfunction, immune abnormality, and increased incidence of cancer. Recently, the effect of endocrine disrupters on the human body has drawn attention as a social issue. Bisphenol A [BPA, 2,2-bis(4-hydoxyphenyl)propane] is a chemical produced by the combination of two phenol radicals and one acetone molecule. It is an endocrine-disrupting chemical and its effects on the human body are of great concern. The toxicities of BPA include reproductive toxicities [1, 2]. When pregnant female mice were fed BPA, their male offspring had a decreased sperm count per day, decreased ability to generate sperm, and a prostate with increased weight [3].

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Kazutaka Murakami
1-98 Dengakugakubo Kutsukake-cho
Toyoake 470-1192 (Japan)
Tel. +81 562 939 245, Fax +81 562 931 830
E-Mail nephrokm@fj9.so-net.ne.jp

When male rats were exposed to BPA, testosterone production was suppressed in association with decreased LH-β and increased estrogen receptor-β pituitary mRNA levels [4]. There have been several reports on the carcinogenicity [5], chronic toxicity [6, 7] and the effect of BPA on the immune system [8] of rats and mice. In addition, some reports have demonstrated the effect of BPA on bone metabolism [9, 10]; BPA increased alkaline phosphatase activity and enhanced bone mineralization in a mouse calvaria osteoblast-like cell line [9]. BPA could be eluted from dentin-bonding agent [11, 12]. The effects of BPA on the human body are still awaited to be evaluated. BPA is contained in polycarbonate that is used both in hollow fibers and in the housing or potting of dialyzers.

In the present study, we eluted BPA from hollow fibers made of various materials, and also measured the accumulation of BPA in the sera of patients with renal failure.

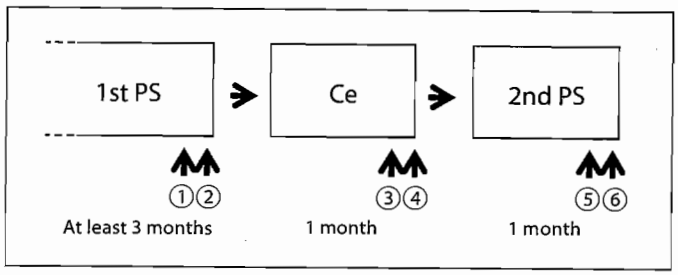

Fig. 1. Method (experiment 2). Cross-over test among patients who were undergoing chronic hemodialysis. 15 patients underwent hemodialysis with a PS dialyzer for at least 3 months, followed by hemodialysis with a Ce dialyzer for 1 month, and then again hemodialysis with a PS dialyzer. Blood samples were obtained before and after three hemodialysis sessions (↑). Blood samples were obtained on a Monday or Tuesday at 4-week intervals, and the serum BPA concentration was determined: ① = pre-HD (PS-dialyzer), ② = post-HD (PS-dialyzer), ③ = pre-HD (Ce-dialyzer), ④ = post-HD (Ce-dialyzer), ⑤ = pre-HD (PS-dialyzer), ⑥ = post-HD (PS-dialyzer).

Patients and Methods

Experiment 1

We performed experiments in vitro using hollow fibers taken out of individual dialyzers as experimental materials. We used hollow fibers made of the following materials: polymethylmethacrylate (PMMA) (Toray Co., Ltd, BK-U, Tokyo, Japan), polysulfone (PS) (Fresenius Kawasumi, Co., Ltd, PS-UW, Tokyo, Japan), cellulose triacetate (CTA) (Nipro Co., Ltd, FB-U, Osaka, Japan), cellulose (Ce) (Asahi Kasei Medical Co., Ltd, AM-BC, Tokyo, Japan) and polyester-polymeralloy (PEPA) (Nipro Co., Ltd, FLX, Osaka, Japan). 10 mg of the hollow fiber were frozen, crushed and dissolved in hexane. After evaporating the solvent, the residue was redissolved in dimethylsulfoxide. The concentration of BPA was measured by enzyme-linked immunosorbent assay (BPA ELISA Eiken; Eiken Chemical Co., Ltd, Tokyo, Japan).

The sensitivity of this kit for BPA was >0.3 ng/ml. Six samples of each material were tested.

Experiment 2

The serum BPA concentration was measured in 22 patients (14 men and 8 women; age 64.7 ± 13.1 years (mean ± SD)) with renal disease who had not undergone hemodialysis. In 6 of the 22 patients, the serum creatinine level was <1.0 mg/dl and the glomerular filtration rate (GFR) assessed by the MDRD method was >90 ml/min, and they were considered to have normal renal function. In addition, we measured the serum BPA concentrations in 15 patients (8 men and 7 women) who were undergoing hemodialysis chronically at our hospital. Their mean age was 69.1 ± 8.4 years and the mean period during which they had undergone hemodialysis was 8.44 ± 2.77 years. Their general condition was stable. Although the patients took usual meals every day, they took no meals just before and during hemodialysis. In addition, the BPA concentration was measured before and after hemodialysis sessions, accompanied by a 1-month crossing over between using Ce (Asahi Kasei Medical Co., Ltd) and PS dialyzers (Fresenius Kawasumi, Co., Ltd) (fig. 1). Prior to commencement of this study, all 15 patients had undergone hemodialysis for at least 3 months with the same PS dialyzer examined in this study. All blood specimens were collected before and after hemodialysis was carried out at the beginning (Monday or Tuesday) of the week immediately before a series of dialyses with one type of dialyzer during 1-month crossing over was concluded. Serum BPA concentrations before and after hemodialysis with one or the other of the dialyzers were compared. In addition, the serum BPA concentrations before and after dialysis with the PS dialyzer were compared with those before and after dialysis with the Ce dialyzer.

All values were expressed as the mean ± SD of duplicated experiments. Data were evaluated statistically with Student's t test. A value of $p < 0.05$ was considered to be statistically significant.

Results

Experiment 1

BPA was eluted from all tested hollow-fiber materials. In particular, a large amount of BPA was eluted from PS and PEPA hollow fibers, in which the BPA concentration was 83.8 ± 5.1 and 122.5 ± 6.1 ng, respectively (fig. 2). A significantly larger amount of BPA was eluted from PEPA than from PS hollow fibers ($p < 0.01$), and significantly larger amounts of BPA were eluted from PS and PEPA hollow fibers than from hollow fibers made of other materials ($p < 0.01$). Figure 2 shows the comparisons with other hollow-fiber materials.

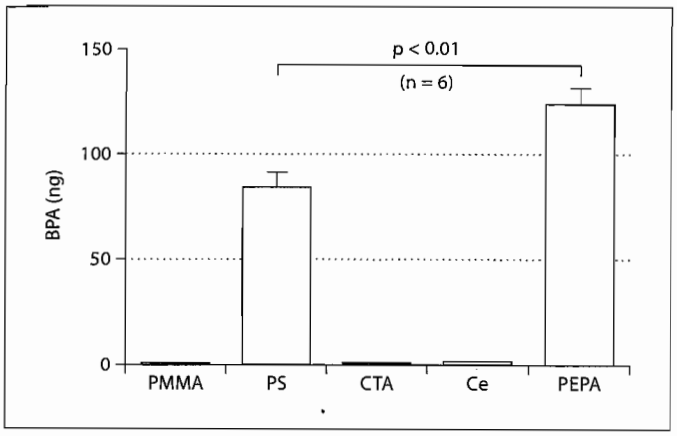

Fig. 2. Amount of BPA eluted from hollow fibers made of various materials. Data are shown as mean and SD (n = 6). PMMA = polymethylmethacrylate; PS = polysulfone; CTA = cellulose triacetate; Ce = cellulose; PEPA = polyester-polymeralloy. Amounts of BPA eluted from PMMA, PS, CTA, Ce, and PEPA were 0.08 ± 0.01 ng, 83.8 ± 5.1 ng, 0.08 ± 0.01 ng, 0.16 ± 0.02 ng and 122.5 ± 6.1 ng, respectively.

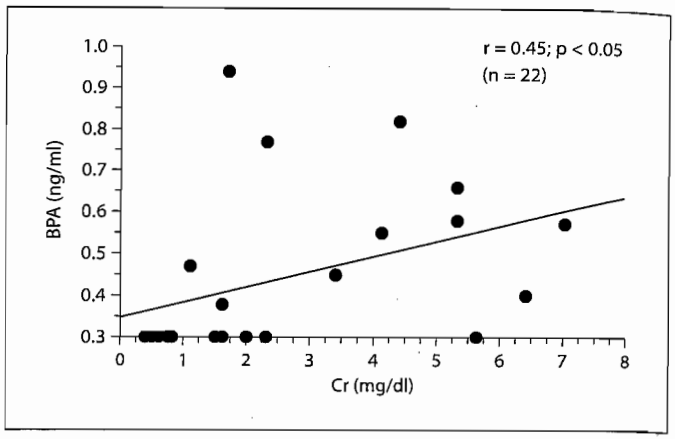

Fig. 3. Correlation of serum BPA concentration and serum creatinine concentration among the 22 patients with renal disease who had not undergone hemodialysis.

Experiment 2

In the 22 patients with renal disease who had not undergone hemodialysis, the serum BPA level was 0.40 ± 0.23 ng/ml. The serum BPA level increased as renal function deteriorated. The 6 patients who had normal renal function (i.e., Cr level <1.0 mg/dl and GFR (assessed by the MDRD method) >90 ml/min/1.73 m^2) did not have a detectable level of BPA (no more than 0.3 ng/ml). A significant positive correlation was observed between BPA level and serum creatinine among the 22 patients ($r = 0.45$; $p < 0.05$) (fig. 3). A significant negative correlation was noted between the BPA level and GFR evaluated by the MDRD method ($p < 0.05$).

Fifteen patients who had undergone chronic hemodialysis with a PS dialyzer for at least 3 months underwent the cross-over test (fig. 1). The serum BPA level increased significantly after dialysis with a PS dialyzer, from 4.83 ± 1.94 to 6.62 ± 3.09 ng/ml after dialysis with a PS dialyzer (1st) and 3.78 ± 2.57 to 4.27 ± 2.98 ng/ml after dialysis with a PS dialyzer (2nd). On the contrary, the serum BPA level decreased from 2.01 ± 2.10 to 1.48 ± 1.41 ng/ml after dialysis with a Ce dialyzer, although there was no significant difference. In addition, the serum BPA concentrations before and after hemodialysis were significantly higher in patients using a PS dialyzer than in those using a Ce dialyzer (fig. 4). The BPA concentration in the 15 patients who were undergoing hemodialysis regularly was significantly higher than that in the 22 patients who were not undergoing dialysis ($p < 0.01$). No significant correlation was observed between the BPA level and the period during which individual patients had been treated with dialysis.

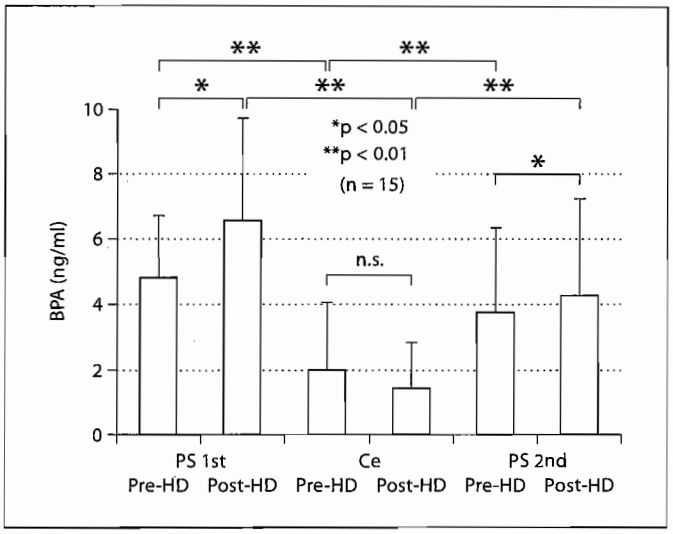

Fig. 4. Comparison of serum BPA concentrations pre- and post-hemodialysis sessions for PS and Ce dialyzers in the cross-over test among patients who were undergoing chronic hemodialysis. BPA levels in serum increased significantly, from 4.83 ± 1.94 to 6.62 ± 3.09 ng/ml after dialysis with a PS dialyzer (1st) and 4.09 ± 2.78 to 4.27 ± 2.98 ng/ml after dialysis with a PS dialyzer (2nd). On the contrary, serum BPA levels decreased from 2.01 ± 2.10 to 1.48 ± 1.41 ng/ml after a session of dialysis with a Ce dialyzer. All values are expressed as the mean ± SD (n = 15).

Discussion

BPA, which is mainly contained in polycarbonate, epoxide resin, and polyarylate, is considered to be an endocrine-disrupting chemical that acts mainly as a female hormone. When BPA enters the body, BPA is initially lipid-soluble. As long as BPA remains soluble in lipid, it has toxicity as an endocrine-disrupting chemical. Once it becomes water-soluble after glucuronization in the liver, however, its toxicity decreases.

In rats, BPA is transformed to glucuronide in the liver, and thereafter the BPA is excreted in the bile to enter the enterohepatic circulation [13]. In humans, orally ingested BPA would not accumulate in the body because it is rapidly conjugated in the liver to a water-soluble form and excreted in the urine and partly in the feces, within 18 h after systemic absorption [14]. Therefore, the possibility of BPA accumulation might be small if renal function is normal.

Although there are a few reports on the accumulation of BPA in patients with renal dysfunction, the numbers of cases studied were very small [15, 16].

In the present study, the serum BPA concentration was determined in a larger number of renal disease patients who were either on or not yet on hemodialysis. The serum BPA concentrations found in these patients were higher than those found in healthy subjects reported previously [17]. BPA was not detected in 6 subjects with normal renal function in the present study. The BPA level in non-dialysis renal failure patients increased in parallel with the serum creatinine level. This positive correlation between serum creatinine and BPA demonstrates that deterioration of renal function may cause accumulation of BPA.

The ELISA kit used in our study measures mainly lipid-soluble BPA, but has partial cross-reactivity with the water-soluble form [17]. The cross-reactivity of the lipid-soluble and water-soluble forms is 100 and 56.6%, respectively. Therefore, water-soluble BPA may also have been measured in the current study.

Thus, the increased serum BPA concentration in renal disease patients who had or had not undergone hemodialysis could partially have been due to the measurement of water-soluble BPA as well as lipid-soluble BPA.

Some hollow fibers used in hemodialyzers are known to contain BPA, and there might be concern about the possibility of BPA elution [18, 19]. The present in vitro study revealed marked elution of BPA in hollow fibers made of PS and PEPA, which consist of raw materials containing BPA. Furthermore, the in vivo study showed that the BPA level was significantly higher in hemodialysis patients than in patients with renal failure without hemodialysis ($p < 0.01$), particularly when the dialysis device was made of PS.

The BPA level, however, was also increased in patients with renal failure without hemodialysis since these patients had impaired excretion of BPA. Also, in patients who undergo hemodialysis with hollow fibers containing BPA, the serum BPA level markedly increases further due to both elution of BPA from the hollow fiber and delay in its metabolism.

Even when the dialysis apparatus was made with the Ce dialyzer, in which the hollow fibers did not contain BPA, the serum BPA concentration in the patients was higher than that in patients who did not undergo hemodialysis. This observation might be related to the fact that almost all patients in this study had anuria, and consequently excretion of BPA was prevented. It was also found that the serum BPA concentration tended to decrease after dialysis with the Ce dialyzer, although there was no significant difference. This tendency might have resulted from the fact that BPA could be removed during dialysis with the Ce dialyzer, because BPA has a molecular weight of 228.3 kDa.

The maximum tolerated amount of BPA taken orally is 0.05 mg/kg/day according to the Food Sanitation Law of Japan. When the weight of the patient is assumed to be 50 kg, the tolerated amount of orally-administered BPA is 2.5 mg/day. Since about 5% of orally-administered BPA enters into the circulating blood, about 125 μg of the 2.5 mg BPA taken orally enters the bloodstream [13]. When a patient undergoes hemodialysis once with a PS dialyzer, about 8 μg of BPA is considered to enter into the blood on the assumption that BPA does not enter into other bodily partitions. This amount cannot be ignored in view of the additional oral intake of BPA and also the fact that the metabolism of BPA slowed down in patients who required hemodialysis.

When BPA-containing hollow fibers are used over a long period of time, BPA is considered to persistently and continuously migrate to the serum and accumulate there since elimination of BPA is delayed in these patients with deteriorated renal function. In addition, the BPA that leaks from the hollow fibers passes through the heart and lungs via a venous shunt and enters the general circulatory system. Therefore, metabolism in the liver could be delayed when compared to that after BPA ingestion. Lipid-soluble BPA has the potential to be distributed to multiple organs. As a result, there is concern that BPA in the state of lipid solubility may accumulate in body tissue without being metabolized in the liver. Furthermore, in

tissues, BPA could accumulate in more concentrated amounts compared to that in serum [20].

It is assumed that a large amount of BPA actually enters the patient's body during a hemodialysis session. When using the dialyzer made of PS, the BPA level in the serum increased after the hemodialysis session. It is difficult to assess the risk in patients with chronic exposure to small amounts of BPA [21]. Also, the maximum safe level of BPA in the serum is still not known.

Since BPA accumulation could affect the endocrine or metabolic system of hemodialysis patients, further investigations on these issues are necessary.

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