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Original Article

Daily Urinary Excretion of Bisphenol A

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ABSTRACT

Objectives: Concerns over dietary exposure to bisphenol A (BPA), an endocrine disruptor, have been raised because BPA is contained in resins and plastics commonly used for the preservation of food and beverages. The purpose of the present study was to assess daily intake levels of BPA in a group of male subjects by measuring total urinary BPA (free BPA plus BPA released by treatment with β-glucuronidase), as well as determining intra-individual variation in BPA excretion.

Methods: Twenty-four-hour urine was collected from 5 subjects for 5 consecutive days for the evaluation of between-day variation in urinary BPA excretion and from 36 male subjects for the estimation of the level of daily BPA intake. BPA in the urine samples was measured by GC/MS/MS following enzymatic hydrolysis of BPA glucuronate, solid phase extraction, and derivatization.

Results: A large between-day variation was found over 5 days for the daily excretion of urinary BPA in the 5 subjects. The daily excretion of urinary BPA was distributed log-normally in the 36 male subjects, with the median value being 1.2 μg/day (range: <0.21-14 μg/day), which was far below the Tolerable Daily Intake (0.01 mg/kg bw) recommended by a scientific committee in the European Commission in 2002. However, the maximum estimated intake per body weight (0.2 μg/kg/day) was only one order of magnitude lower than the reported lowest level for reproductive/behavioral effects in pregnant mice (2 μg/kg/day).

Conclusions: Measuring urinary BPA in urine is a suitable approach for estimating short-term BPA intake levels in individuals and/or estimating the average exposure level of populations. Urine analyses will be increasingly important in the human health risk assessment of BPA.

Key words: bisphenol A, 24-h urine, daily excretion level, daily intake, intra-individual variation

Introduction

The estrogenic activity of bisphenol A (2,2-bis (4-hydroxyphenyl)propane: BPA) was first reported in 1938 by Dodds et al. (1). In 1993, BPA attracted attention as a typical endocrine disruptor after Krishnan et al. (2) reported its proliferative activity in human mammary cancer cells (MCF-7). A Tolerable Daily Intake (TDI) for BPA of 0.01 mg/kg/day, based on the no-observed-adverse-effect-level (NOAEL) of weight loss in rats, was subsequently recommended by a scientific committee in the European Commission in 2002 (3). Several studies, however, have shown reproductive effects in the offspring of pregnant mice exposed to BPA at levels (2 to 20 μg/kg/day) comparable to the proposed TDI (4-10). Although these *in vivo* and *in vitro* studies demonstrated that low doses of BPA cause adverse health effects, the effect of BPA exposure on human health has not yet been evaluated.

Exposure assessment is an essential part of the health risk assessment of a chemical. Because BPA is contained in polycarbonate plastics and epoxy resin, which are used for containers and the lining of cans for food and beverages, it is probable that people are exposed to BPA through foodstuffs and beverages preserved in this way. For example, Kawamura et al. (11) reported that a canned coffee beverage contained 40 μg of BPA per can, and Ozaki et al. (12) reported 26 μg/g of BPA in reclaimed paper used for food wrapping. Quantitative estimates of human exposure to BPA, however, are limited, and more data are needed to evaluate the possible health risk.

In contrast to the limited information on BPA exposure

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levels, the metabolism of BPA in humans and monkeys has been well described (13, 14). BPA is efficiently absorbed (closed to 100%) from the digestive tract, and then rapidly biotransformed to a glucuronide metabolite which is excreted in the urine. The overall biological half-life of BPA in humans is less than 6 h (14), and thus urine analysis is suitable for assessing recent exposure to BPA.

Most studies determining urinary BPA in urine carry out the analysis on spot urine samples (15-20). However, the short biological half-life of BPA could cause considerable within-day variation in subjects, and, hence, results from spot urine analyses may not provide a reliable estimation of daily BPA intake. The purpose of the present study was to assess daily intake levels of BPA in a group of 36 young men by measuring the quantity of urinary BPA in 24-h urine samples. In addition, intra-individual variation in BPA intake was examined by measuring urinary BPA in 24-h urine samples collected over 5 consecutive days from 5 adults.

Materials and Methods

Chemicals

Standard solutions of BPA (Tokyo Kasei Kogyo, Tokyo, Japan) were prepared in acetone. The d-16 BPA was obtained from Cambridge Isotope Laboratories (Massachusetts, USA) and the β-glucuronidase solution (*E-coli*, 200 U/ml) was from Roche Biochemical Diagnostics (Mannhein, Germany). Acetone, hexane, dichloromethane, ethyl acetate, methanol and N, O-bis (trimethylsilyl) trifluoro acetamide (BSTFA) was obtained from Wako Pure Chemicals (Osaka, Japan). Solid phase extraction was performed with an NH2 SPE column (6 ml) obtained from International Sorbent Technology (Mid Glamorgan, UK); the column was conditioned with acetone (10 ml) followed by hexane (2×10 ml) prior to use (21).

Materials

Glassware was cleaned by washing with detergent and tap water, and rinsing with purified water (Milli-Q SPTOC from Nihon Millipore, Tokyo, Japan), followed by heating at 200℃ for 4 h. Volumetric glassware was cleaned by the same procedure except that it was rinsed with methanol instead of being heated in the last step. Polypropylene (PP) bottles (500-ml and 2-l capacity), cleaned by the same procedure described for the volumetric glassware, were used for urine collection. Gas chromatography/ ion trap tandem mass spectrometry (GC/MS/MS) was performed with a Polaris Trace GC from Thermo Electron Co. Ltd (California, USA). Table 1 shows the analytical conditions used for the GC/MS/MS analyses.

Table 1 Analytical conditions for GC/MS/MS

Column	HP-5MS	
	30 m×0.25 mm. i. d. df=0.25 μm	
Column Temperature	60°C (2 min)→10°C/min→260°C	
Injection Temperature	250°C	
Carrier gas	Helium 1.2 ml/ml	
Injection	3 μl/Injection pulsed splitless mode	
Interface Temperature	260°C	
Ion source Temperature	200°C	
Ion measured for quantitation		
Retention Time (min)	BPA	20.38
	BPA-d16	20.31
Monitoring ion	BPA	m/z 357→m/z 191, 267, 341
	BPA-d16	m/z 368→m/z 197, 277, 348

Analytical methods

A portion (1.0 ml) of the urine sample was transferred to a glass centrifuge tube and the β-glucuronidase solution (50 μl) was added. After incubation (37℃ for 120 min), d-16 BPA (10 ng) and dichloromethane (2.0 ml) were added to the sample tube and the mixture was manually shaken and then centrifuged (1500 rpm for 20 min). A portion (~ 1.5 ml) of the dichloromethane layer was transferred to a new glass centrifuge tube and the solvent was evaporated under a stream of nitrogen. The residue was dissolved in hexane (2 ml) and loaded onto a preconditioned NH2 SPE column. The column was washed with a mixture of dichloromethane: hexane (8 ml, 1:1), followed by a mixture of dichloromethane: ethyl acetate (8 ml, 1:1). The BPA was then eluted from the column with acetone (8 ml). The acetone wash was transferred to a new glass centrifuge tube, the solvent evaporated by a stream of nitrogen, and the residue redissolved in acetone (100 μl). An aliquot (100 μl) of BSTFA was added to derivatize the BPA before analysis by GC/MS/MS. Figure 1 shows a flow diagram of the sample preparation steps (21).

Urine samples

Sampling

Each subject was asked to void urine into a precleaned 500-ml PP bottle. The contents of the bottle were then transferred to a 2-l PP bottle which was stored on ice. Urine was collected in this manner over a 24-h period, after which time the cumulative urine sample was thoroughly mixed, its volume recorded, and a portion (~15 ml) was removed and stored frozen for later BPA measurements.

Intra-individual variation (between-day variation)

Twenty-four-hour urine was collected from 5 healthy

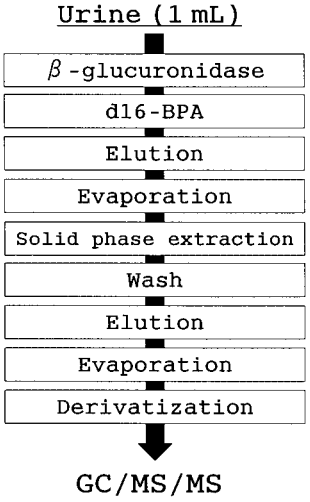

Fig. 1 Flow diagram for the sample preparation procedure.

adults (4 males and 1 female; age, 22-40 yrs) for 5 consecutive days in May 2002 for the assessment of intra-individual variation in the daily excretion of urinary BPA.

Daily urinary excretion level

Twenty-four-hour urine was collected from 36 male subjects (university students from the University of Tokyo) with a mean age of 24.7±3.0 yrs from April to July, 2003.

The purpose and outline of the present study were explained to all of the subjects and urine sampling was carried out after oral informed consent was obtained.

Results

Urine analysis by GC/MS/MS

The detection limit for BPA in the urine samples, defined as three times the standard deviation of blank measurements, was 0.38 ng/ml. The repeatability of the analysis was 9% (n=5) when expressed as relative standard deviation (RSD). Recovery of the added standard solution of BPA (10 ng) to 4 urine samples ranged from 62 to 124% with the mean being 87%.

Intra-individual variation

Figure 2 shows the daily urinary BPA excretion by the 5 healthy adults for 5 consecutive days. BPA was detected in 3 of the 5 subjects and its daily excretion ranged from <0.58 to 13 μg/day (median 1.3 μg/day). Intra- and inter-individual variation in the excretion was calculated to be 91% and 84%, respectively, by the analysis of variance.

Daily urinary excretion level

Figure 3 shows the histogram of daily urinary BPA excretion of the 36 male subjects. BPA excretion ranged from <0.21 to 14 μg/day (median 1.2 μg/day), corresponding to <0.003 to 0.23 μg/kg/day (median 0.02 μg/kg/day).

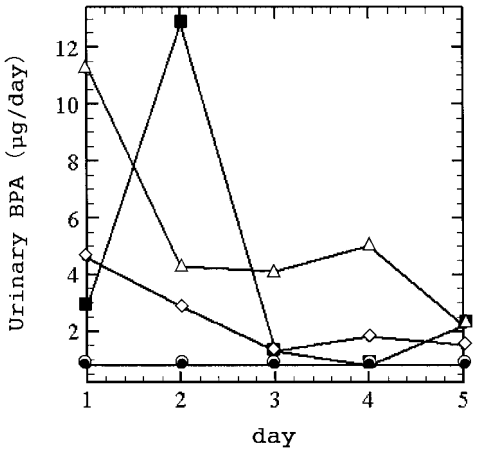

Fig. 2 Daily inter- and intra-individual variation in the excretion of urinary BPA for 5 individuals over a 5-day period. Different marks in the figure indicate different subjects. The not-detectable samples are plotted as half of the detection limit value (0.58 μg/day).

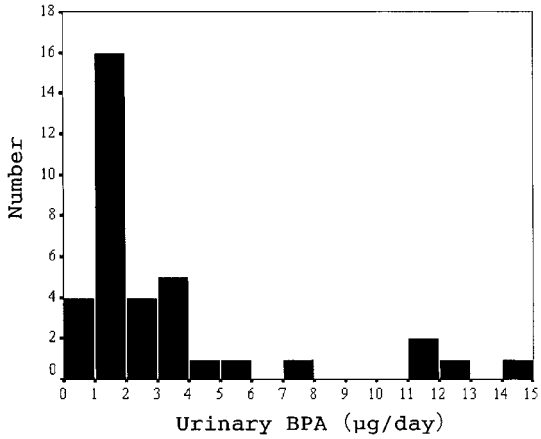

Fig. 3 Histogram of the daily excretion of urinary BPA (μg/day) in 36 male subjects.

Discussion

The analytical method used in this study was originally developed for the analysis of BPA in environmental waters (21). The recovery (average 87%) and repeatability (RSD 9% at 5 ng/ml) obtained in the current study showed that the method is also applicable to urine analysis.

The daily urinary BPA excretion by the 5 healthy adults for 5 consecutive days showed large intra- and inter-individual variation (Fig. 2), indicating that even 24-h urine sampling does not allow us to assess quantitatively the differences in BPA excretion between individuals. Because the half-life for BPA is <6 h (14), the level of urinary BPA found in the 24-h urine samples can be assumed to represent the BPA intake on the day of sampling and/or on the day before sampling. Since the analysis of 24-h urine can reduce the within-day variation in the analyte concentration caused by the variation in the time lag between intake and sampling, the results obtained from such samples should indicate daily intake better than the results obtained from spot urine samples. The large variation in daily urinary BPA excretion, based on the 24-h urine analysis, indicates that daily BPA intake also varies widely. Thus, measurement of urinary BPA in 24-h urine should not be used to estimate the long-term BPA intake of a subject if the frequency of urine sampling is limited. It remains possible, however, that 24-h urine analyses can be effective in estimating the average BPA intake of a population when a cross-sectional study is performed.

The daily excretion of urinary BPA by the 36 male subjects of our study ranged from <0.21 to 14 μg/day (median 1.2 μg/day) (Fig. 3). Three previous studies (16-18) carried out in Japan on BPA excretion reported the BPA concentrations in spot urine. In order to compare those results with our own data, we estimated the daily urinary BPA excretion from the reported concentration data by assuming that daily urine excretion volume was 2 l, and/or that daily urinary creatinine excretion was 1.2 g (18). Imai and Morita (16) found 0.12 to 4.6 μg/day in 12 subjects, and Hanaoka et al. (17) found a median value of 2.5 μg/day excretion in workers exposed to diglycidyl ether of BPA (control subjects excreted 1.2 μg/day). Ouchi and Watanabe (18) reported a median urinary concentration of 0.77 ng/mg creatinine, corresponding to 0.92 μg/day excretion in 48 female

subjects. These three sets of data, based on spot urine analyses, indicate that the excretion of urinary BPA by Japanese populations ranges from 0.1 to 14 μg/day with median values between 0.92 and 1.2 μg/day. The median values for spot urine samples were close to the values (median 1.2 μg/day) we obtained for 24-h urine samples and the range was also comparable. Recently, Tsukioka et al. (22) reported that BPA excretion in 24-h urine for 22 subjects ranged from 0.48 to 4.5 μg /day (mean±SD 1.7±1.3 μg/day), which was also comparable to our estimate.

Imanaka et al. (23) analyzed BPA concentrations in hospital meals and estimated a BPA intake of 0.19 to 3.7 μg/day (mean 1.3 μg/day). Since excretion of ingested BPA is almost complete in 24 h (14), the quantity of BPA in 24-h urine approximately equals the quantity ingested during the previous 24 h. Therefore the daily urinary excretion of 1-2 μg/day BPA obtained in our investigation and in related studies (16-18) can be regarded as the average daily intake of BPA. This exposure level is comparable with the estimate by Imanaka et al. (23). Thus, all available data indicate that, on average, the Japanese consume 1-2 μg of BPA daily. Interestingly, this level of exposure is considerably less than the 10.7 μg BPA/day reported for Koreans (20). The Korean exposure level was calculated from the geometric mean urinary level (8.91 μg BPA/g creatinine) of 73 subjects with an estimated daily creatinine excretion of 1.2 g.

Comparison of our data with those reported by Tsukioka et al. (both are based on 24-h urine) revealed that the distributions were different: our data were skewed toward higher excretion approximating a log-normal distribution (Fig. 3), while the data of Tsukioka et al. appeared to be normally distributed covering a smaller range of values (0.48 to 4.5 μg/day). This might reflect the different populations in the two studies; university students (average age of 24.7 yrs) were used in our study while older people (average ages of 48 yrs for males and 41 yrs for females) were the subjects in the study of Tsukioka et al. (24). The difference might be related to lifestyle, including food consumption patterns. The subjects of our study provided details about the type of food they consumed on the day of, and on the day before, the sampling of 24-h urine; we could not, however, find any association between the excretion of urinary BPA (and, hence, ingestion of BPA) and the type of food item consumed. A more detailed study is required in order to identify the foodstuffs responsible for elevated urinary BPA excretion.

The maximum estimated intake per body weight (0.23 μg/kg/day), calculated from the present urinary BPA analysis of the 36 subjects, was well below the TDI recommended by a scientific committee in the European Commission (3). However, there are several reports of reproductive and behavioral effects observed at concentrations close to the proposed TDI, or at even lower doses. Sakaue et al. (10) reported decreased spermatogenesis in adult mice exposed at 20 μg BPA/kg/day. The research group of vom Saal studied the effects on the offspring of pregnant mice exposed to low doses of BPA (2-2.4 μg/kg/day), and reported decreased sperm production and increased prostate weight in the males, and advanced puberty in the females (4-7). The same group of researchers also found deterioration of maternal behavior in mice exposed to 10 μg/kg/day BPA (8). Kawai et al. (9) recently reported aggressive behavior and decreased testis weight in male offspring of pregnant mice exposed to BPA at 2 μg/kg/day. In additional to adverse physiological effects, functional effects were also found in these two studies (8, 9). However, it must also be noted that some of these low-dose effects were not reproduced in other studies (25-27). In conclusion, we note that the estimated maximum daily intake of BPA per body weight (0.23 μg/kg/day) found in our study with humans is only ten-fold lower than doses shown, in several recent investigations, to produce adverse effects in animals. Our preliminary data indicate that more studies of human exposure to BPA are needed, e.g., evaluation of the BPA intakes of other populations with different characteristics, to fully evaluate the possible health risks. We expect that urine analyses will play an important role in future studies.

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