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Profiles of urinary bisphenol A in human panel during an exposure intervention

노출 중재 동안 소변 중 비스페놀 A의 프로파일 연구

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Abstract

Profiles of urinary bisphenol A in human panel during an exposure intervention

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Bisphenol A (BPA) is a chemical commonly used in consumer products of polycarbonate plastics, epoxy resin cans and thermal ingredient of receipt paper such as food or beverage containers, thermal receipts, and medical devices, etc. Several researches in toxicology and epidemiology have been indicated that exposure to BPA may be associated with a variety of adverse outcomes, although the evidence for low dose effects is less certain. Some of recent studies

reported use of polycarbonated containers, canned drinks or thermal paper could contribute to increase of body burden with estimated exposure amounts, but there were limitations of uncertainty coming from application of limited pharmacokinetic (PK) information (i.e. urinary excretion fraction from few subjects) or exposure models. The aim of the present study was to measure contribution by exposure source in daily life and provide another PK-based approach to exposure amounts. Four adult males were exposed to BPA via use of polycarbonated container, commercial canned drinks and thermal paper and three matched volunteers participated with avoidance of the exposure sources of interest at the same place as in the exposed. All urine samples were collected and combined to monitor. 5 hours before the exposure as well as at 0.5, 1, 2, 4, 6, 8, 12 and 24 hr. Urinary BPA were measured with liquid chromatography coupled with tandem mass spectrometry and the intake amounts of BPA were estimated with a human two-compartment PK model for oral consumption of BPA, which emulated about 70% of first exposed amounts in 6 hours after exposure with 3.42 hours for the biological half-time. Cumulative amounts of BPA until 6 hours, were significantly higher in users of polycarbonate bottle compared to the control (p = 0.029); however, those for drinking of canned beverage and paper crafts of thermal paper did not make significant contribution. Based on urinary amounts of BPA, single drinking 200 mL of purified water with polycarbonated cup, single drinking of 200 mL canned

beverage and paper craft with thermal paper (9 ×15 cm^2 for 15 min) contributed to about 0.7~2.6 μg, 0.3~2.1 μg and 0.1~1.5 μg of intake amounts of BPA, respectively. Although the exposure experiment was performed in a confined and planned design, there was a relatively wide inter-personal variability in the urinary BPA measures and intake amounts. Considering uncontrolled spontaneous exposure to BPA in daily life, further studies are required to explore the variability in the environmental exposure and personal kinetics, but the present study showed single use of consumer products of BPA could cause distinguishable contribution to body burden.

Keywords: Bisphenol A, human exposure experiment, intake amounts, consumer products, pharmacokinetics

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Ⅰ. Introduction

Bisphenol A (BPA) is a chemical commonly used in products of polycarbonate plastics, epoxy resin cans and thermal ingredient of receipt paper. Polycarbonate plastics are used extensively in consumer products including food containers, thermal paper, and medical devices; general population exposure may also occur in drinking water via BPA in water pipes and storage tanks (Christensen et al., 2012). Detectable levels of BPA have been reported in most human biological samples from numerous studies worldwide. Such widespread exposure is of concern given the evidence from toxicology and epidemiology studies that exposure to BPA may be associated with a variety of adverse outcomes, although the evidence for low dose effects is less certain (Braun and Hauser, 2011; Hengstler et al., 2011).

BPA is endocrine-disrupting chemical that can affect in a number of organ systems. BPA shows affinity for the estrogen receptor and may alter its function by blocking or mimicking the action of estrogen (Hiroi et al., 1999). Previous epidemiological studies have reported the associations between BPA exposure and adverse health effects on the reproductive (Cantonwine et al., 2010) and endocrine systems (Lang et al., 2008).

There is increasing evidence to support wide-ranging health effects from a variety of environmentally relevant exposure sources, pathways, and routes, which are not plausibly predicted from the current risk-assessment approach, even with the application of an uncertainty factor. Because of its extensive use, human exposure to BPA is widespread. Therefore, the European Food Safety Authority (EFSA) reduced the tolerable daily intake to 4 μg BPA/kg/day in 2014.

Orally administered BPA is rapidly metabolized by glucuronidation and sulfation during first-pass metabolism, with a biological half-life of approximately 6 hours and nearly complete elimination within 24 hours (Volkel et al., 2002). BPA exists in low levels in the body, and is removed quickly from the body; however, BPA is high-volume industrial chemical. Therefore, BPA exposure is ubiquitous and has been detected in >90% of the population in the United States (Calafat et al., 2008). There were BPA exposure in different population groups (Becker et al., 2009; Braun et al., 2009; He et al., 2009; Lee et al., 2008).

There were several research that dietary intervention and intervention of major one of BPA exposure sources: polycarbonate plastics and epoxy resin cans. Carwile et al. (2009) demonstrated that the corresponding increase in urinary BPA concentrations after use of polycarbonate drinking bottles and Rudel et al. (2011) reported that urine levels of BPA decreased significantly during the fresh foods intervention, suggesting that most BPA intake came from food packaging

or meals outside the home. The study of Bae and Hong (2015) demonstrated that consuming canned beverage significantly increased the urinary BPA concentration compared with consuming the same beverage in the glass bottle. Additionally, it is necessary to know whether existing the contribution of major exposure sources of BPA in same person and how much the extent of contribution.

Traditionally, urinary concentrations of BPA have been measured from a single spot urine sample, but single spot urine may misclassify BPA exposure for human life pattern (Mahalingaiah et al. 2008). The information about the subject variability of urinary BPA amounts is needed to optimize the design of more elaborated exposure assessment. Thus, we have to realize BPA exposure properties represented personal lifestyle in biomarker.

In the present study, major goals were to measure contribution by exposure source in daily life and provide another PK-based approach to exposure amounts.

Ⅱ. Materials and methods

2.1. Study subjects and BPA treatment

Seven healthy adult males volunteered in August 2013, and four were randomly assigned in exposed group and three in control group (Table 1), where the exposed were exposed to three sources of BPA – polycarbonate bottled water, canned beverage drinks, thermal papers used in general retail shops and the control were not allowed to use, consume nor touch them. The exposure experiment was made by each source for eight hours but monitored for 24 hours, and performed by a week or more interval. The exposed and control subjects stayed at the same place (office room) and spent reading books or using mobile devices (e.g. laptop, smart phone, etc.) except for exposure the test sources. Exposure to the test sources was made twice specifically at zero time (assay starting point) and 6 hours later for polycarbonate bottled water, canned beverage drinks or 4 hours later for the thermal paper. While urine samples were monitored at about 0.5 hours before the exposure and about 0.5, 1, 2, 4, 6, 8, 12 and 24 hours after exposure, all urine samples were collected at other time points were combined based on the check points. During the 8-hr exposure experiments, diet was under the controlled: all participants were provided with

same meal (lunch of rice porridge) in stainless steel container prepared by a commercial dining place.

Specifically for BPA exposure experiment via polycarbonated bottle, the exposed and control subjects drank 400mL of water in polycarbonated bottle and stainless cup, respectively at each exposure. As for BPA exposure via beverage can, the exposed and control drank 400ml of canned beverage (in the same brand and products) and same volume of drinking water in stainless cup, respectively for the first exposure; six hours later, the exposed drank three to five canned of beverage (about 200mL each of various branded products) ad libitum and the control drank same volume of drinking water in stainless cups. For BPA exposure via thermal paper, the exposed made paper craft with three sheets of thermal paper (9×15 cm^2 each) at starting point of the experiment and four hours later. After the 8-hr exposure experiment, all subjects returned back to their usual life and diets but provided all records of diets, drinking and activities. Each subject assigned to exposure and control group was not changed until all exposure experiments finished.

Table 1. Characterization of human subjects participating in the study

Group	Subject	Age (yr)	Height (cm)	Body weight (kg)
Exposure	**A**	23	171	69.5
	B	25	174	85.4
	C	23	170	69.1
	D	44	171	67.7
Control	**E**	25	176	70.5
	F	25	176	65.0
	G	25	182	99.3
Mean ± SD		26 ± 7.5	174.3 ± 4.2	74.3 ± 12.6

[Note] All subjects were male.

Urine samples were collected during the 8-hr exposure experiments by researchers and personally delivered to the lab after briefly stored in their home refrigerator at -20 ^{o}C overnight during the rest of monitoring period. All human subject research activities were conducted in accordance with protocols approved by the Graduate School of Public Health Seoul National University Institutional review board (IRB # GSPHG 01-001).

2.2. Measurement of urinary BPA

To measure the levels of BPA ($C_{15}H_{16}O_2$, MW 228.29) in urine sample, 200 μL of urine and 10 μL of ^{13}C-labeled internal standard was spiked into 15 mL of conical tube, and then 100 μL of 1 M ammonium acetate and 20 μL of β-glucuronidase/sulfatase (Sigma-Aldrich, USA) were added, the enzyme solution was prepared by dissolving 0.4 g of sulfatase (15,275 U/g solid) and 0.00614 g of β–glucuronidase (1,634,000 U/g solid) in 10mL of 1M ammonium acetate buffer solution (pH 5.0), vortexed for 1 minute, incubated at 37℃ for two hours, and then sonicated for 10 minutes. 2 mL of 0.1 M formic acid was added in the sonicated solution. After the solid phase extraction (SPE) cartridges, Oasis™ HLB (Waters, Milford, MA, USA), was compressed with 2 to 3 psi pressure for 1 minute and conditioned with 3 mL of methanol and 3 mL of distilled water subsequently, the samples were loaded to HLB cartridges and then washed with 1 mL of distilled water and 1 mL of 10 % methanol subsequently. After waste tubes were changed into new tubes, samples were eluted with solution with 3 mL of methanol subsequently. The extracts were evaporated under nitrogen stream, reconstituted with 100 μL of 70% methanol, and then transferred to inserts of 1.5 mL amber vials. The levels of BPA were

measured using liquid chromatography – tandem mass spectrometry with an AB Sciex 4000 tandem mass spectrometer (Framingham, MA, USA) coupled to a Shimazu HPLC system (Kyoto, Japan). The Separation was achieved using a Shisheido CAPCELL PAK C18 ACR column (3 µm, 2.0 x 150 mm). The mobile phase composition used in the chromatographic separation was optimized by binary mixtures of 0.1% acetic acid in water (solvent A) and 0.1% acetic acid in acetonitrile (solvent B). Gradient condition was as follow; 0.0-1.0 min, 30 % B; 1.0-2.5 min, 30-45 % B; 2.5-11.0 min, 45-60 % B; 11.0-13.0 min, 60-90 % B; 13-16 min, 90 % B and return to 30 % in 16.1-20 min. The flow rate of the mobile phase was 0.2 mL/min. 10 µL of each sample was injected into the HPLC system. Data acquisition was performed using Analyst 1.5.2 software (AB Sciex, USA).

2.3. Data analysis

2.3.1. Statistical analysis

We performed the statistical analyses using Statistical Analysis Software (SAS), version 9.4 (SAS Institute Inc., Cary, NC). For concentrations below the LOD, we used a value equal to the LOD divided by the square root of 2 (Hornung and Reed, 1990). The urinary BPA amount (urine volume x BPA concentration) followed a log-normal distribution. Thus, before statistical analysis, data of t-test were natural logarithm transformed. The paired t-test was used to compare exposed and control group of cumulative BPA urinary amounts at each exposure experiments. Significance was set at $p < 0.05$. Data were analyzed from first to second exposure for precise analysis by exposure experiments; in polycarbonate bottled exposure, canned beverage exposure and thermal paper, until 6 hours urine, 6 hours urine and 4 hours urine, respectively.

2.3.2. Pharmacokinetic analysis

There were two approaches that we performed estimating BPA exposure intakes using urinary BPA amounts and concentrations. One approach was a pharmacokinetic method using two-compartment model (Figure 1, Table 2). This model consists of value of parameters found through fitting pharmacokinetic data (Volkel et al., 2002) (Figure 2). The simulation software Berkely modonna (ver. 8.3.9, CA, USA). For fitting urinary observations and simulation of the present human exposure experiments, where human pharmacokinetic parameters were optimized from Volkel's report above. For reverse-dosimetry, estimated dose-cumulated urinary BPA amounts chart was constructed (Figure 3) to calculate estimated BPA intakes.

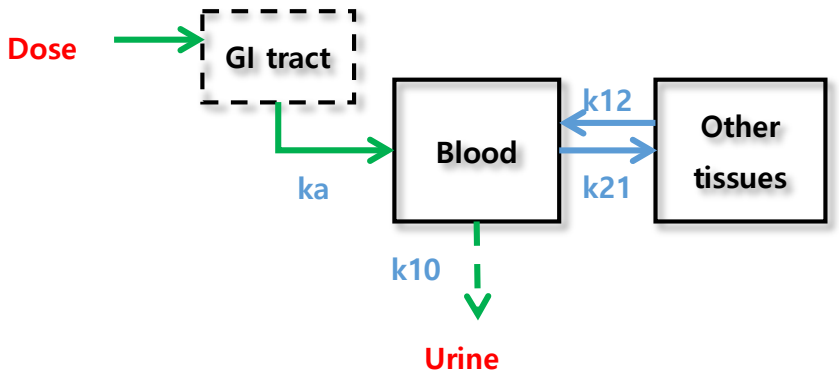


Figure 1. Scheme of two-compartment model with using simulation of urinary elimination kinetics.

Table 2. Parameters and equation used in urinary biomarker modeling

Parameter symbol	Definition	Value	Unit
ka	Rate constant from dose	0.064	/min
k21	Rate constant from other tissues compartment	0.005358	/min
k10	Overall elimination rate constant from blood compartment	0.008169	/min
k12	Rate constant from blood compartment	0.002802	/min
Mw	Molecular weight of BPA	228.29	g/mole

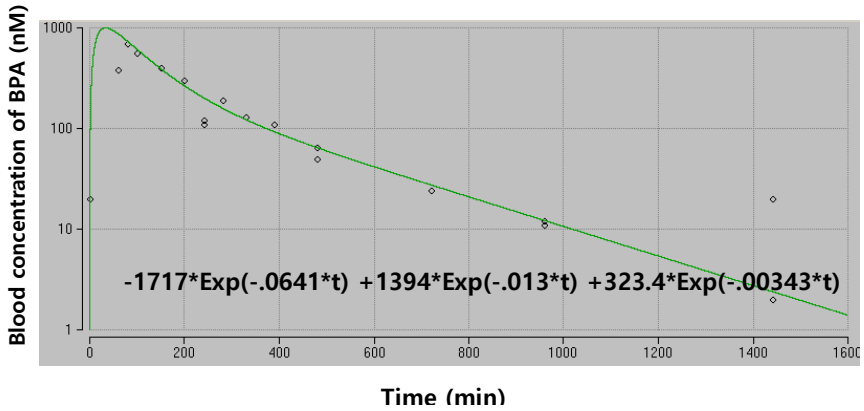


Figure 2. Model validation after fitting to observations in human oral (Volkel et al.,2002).

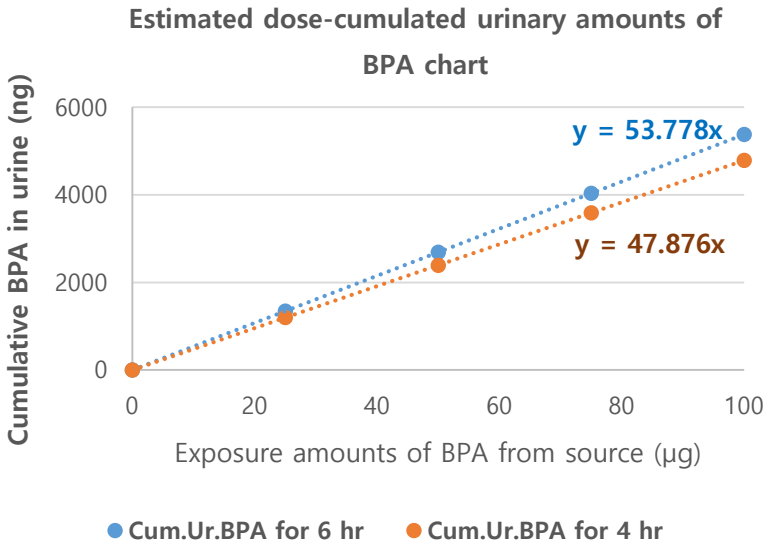


Figure 3. Dose-conversion factor from urinary BPA to environmental exposure (PK simulation for dose reconstruction of BPA).

In other approach, intakes of BPA at first exposure were estimated, using the equation described by Ye et al. (2009), namely:

$$\text{Intakes of BPA during exposure hours (in μg/kg body weight)} = \frac{\text{Concentration BPA in urine (μg/L)} \times \text{Volume of urine (L/exposure hours)}}{F_{ue} \times \text{Bodyweight}}$$

where F_{ue} is the molar fraction of the parent compound excreted as the urinary metabolite (for BPA F_{ue}=1). This simple mathematical equation is widely used.

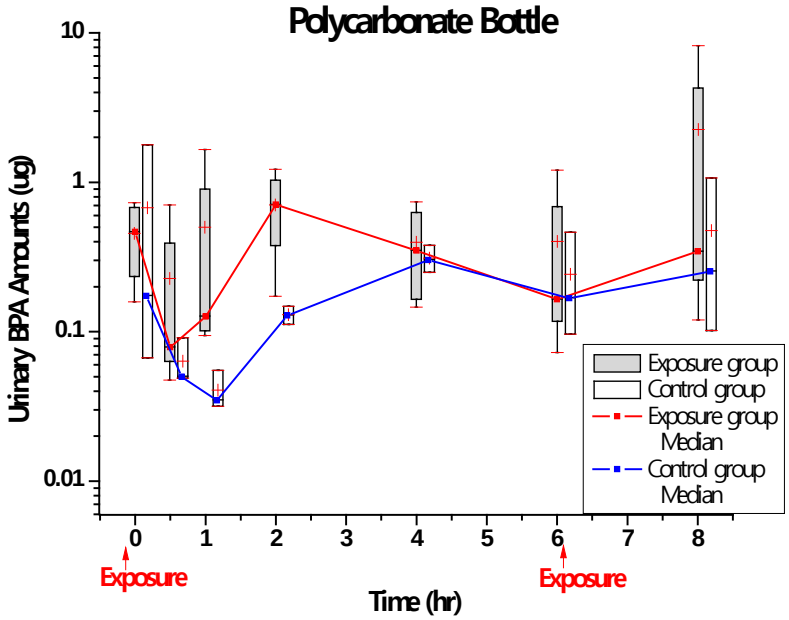
Ⅲ. Results

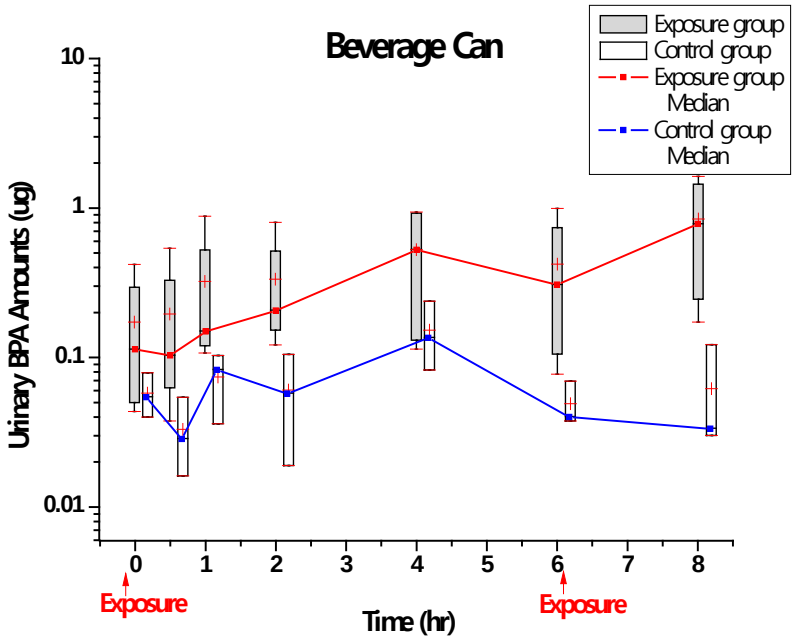
3.1. Time profiles and excretion amounts of urinary BPA by exposure

The urine amount time course showed a day diet trace of participants. Participant's records of eating, drinking and activating could be explained level of urinary BPA amounts (Figure 4). Participant A to D were exposure group, participant E to G were control group. Urine amounts of exposure group tended to have higher levels than control group in between first and next exposure. However, after intervention (> 8 hour), participants went back their normal life and ate each different dinner. Control group also had high levels after eating their general dinner. In particular, participant F and G in control group had same dinner at two days of polycarbonate bottle and beverage can exposure events, their urine amounts increased up to similar levels with same pattern.

We observed comparisons of group and exposure events during intervention. In t-test result, there was significant differences (p = 0.029) of cumulative urinary BPA amounts between exposed and control group for polycarbonate bottle event. Exposure group had significantly higher amounts than did control group. However, among three events in exposure group did not differ

significantly, but urinary geometric mean values of BPA were lower in the order: polycarbonate bottle (2.75 μg) > beverage can (1.37 μg) > thermal paper (0.86 μg). In polycarbonate bottle exposure, cumulative urinary amounts of exposure group was about three times higher than control group (Table 3). In thermal paper exposure, exposure group was about two times higher than control group. Difference between urinary amounts of exposed and control group was calculated to figure out actual intake amounts.


Polycarbonate Bottle
Urinary BPA Amounts (ug)
10
1
0.1
0.01
0
1
2
3
4
5
6
7
8
Exposure
Exposure
Time (hr)
Exposure group
Control group
Exposure group Median
Control group Median


Beverage Can
Urinary BPA Amounts (ug)
10
1
0.1
0.01
0
1
2
3
4
5
6
7
8
Exposure
Exposure
Time (hr)
Exposure group
Control group
Exposure group Median
Control group Median

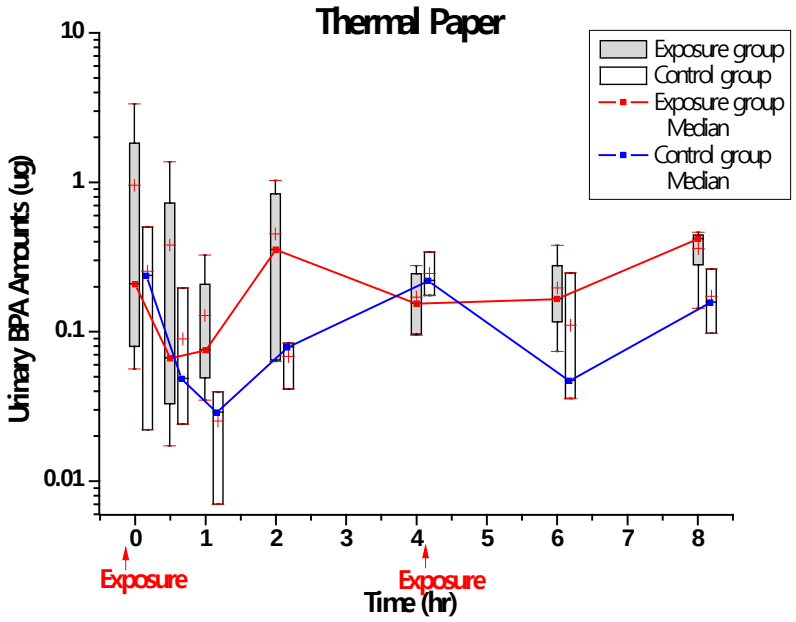


Figure 4. Time profiles of urinary BPA (μg) during the 6-hr exposure experiment by exposure source. Boxes extend from 25th percentile to 75th percentile and the lines indicate median of the urinary BPA for the exposed (n = 4) and the control subjects (n = 3).

Table 3. Cumulative BPA in urine (μg) at monitoring time point after the exposure

		Control group	Exposure group	Fold change
Polycarbonate bottle	Mean	0.77	2.75	3.57**
	(Range)	(0.62 - 1.07)	(1.44 - 5.14)	(2.32 – 4.80)
Beverage can	Mean	0.99	1.37	1.38
	(Range)	(0.44 - 1.65)	(0.51 – 4.15)	(1.16 – 2.52)
Thermal paper[c]	Mean	0.45	0.86	1.91
	(Range)	(0.36 - 0.66)	(0.28 – 3.04)	(NA – 4.61)

Mean: geometric mean; Range: minimum – maximum; Fold change = BPA from the exposed divided by those from the control; **: $p < 0.05$

3.2. Estimation of absorbed amounts of BPA by exposure

3.2.1. Utilization of two-compartment PK model

Difference between urinary amounts of exposed and control group was used estimated dose-cumulated urinary amounts of BPA chart (Figure 3). When 6-hr exposure had assumed that first exposure period, equation was y = 53.778x; y was cumulative urinary BPA amounts. When 4-hr exposure had assumed that first exposure period, equation was y = 47.876x. The median first exposure intake calculated for participants was 46.1 μg (range 15.3 – 75.6 μg) in polycarbonate bottle exposure. 0.26 μg (range 1.47 – 46.48 μg) and 13.0 μg (range NA – 49.68 μg) was in canned beverage and paper craft with thermal paper, respectively. In polycarbonate bottle exposure, estimated intake of exposure group was about three times higher than control group as similar with difference of cumulative urinary BPA amounts.

3.2.2. Utilization of simple fraction of urinary excretion (F_{ue})

The fraction of urinary excretion equation was computed for analysis of urinary data after first exposure, before second exposure. The first exposure intake of BPA was estimated for participants using their urinary BPA amounts. The median first exposure intake calculated for participants was 2.48 μg (range 0.82 – 4.07 μg) in polycarbonate bottle exposure. 0.01 (range 0.08 - 2.50 μg) and 0.62 (range NA – 2.38 μg) was in canned beverage and paper craft with thermal paper, respectively.

3.3. Comparison of estimated intake BPA amounts by exposure

Table 4 shows comparison of estimated intake BPA amounts by 2 approaches during intervention. The estimated minimum, median and maximum intakes of BPA of EIA via F_{ue} through each three exposure sources were 0.82, 2.48 and 4.07 μg, 0.08, 0.01 and 2.50 μg, NA, 0.62 and 2.38 μg, respectively; in the order: polycarbonate bottle, beverage can and thermal paper exposure. To indicate values at once, it was converted units of estimated BPA intakes using two-compartment pharmacokinetic and F_{ue} model. In polycarbonate bottle and thermal paper exposure, estimated intake BPA amounts of F_{ue} model were about twenty times lower than estimated intake using pharmacokinetic model.

Table 4. Comparison of estimated intake amounts (EIA) of BPA by exposure events.

		EIA via PK (μg)	EIA via F_{ue} (μg)
Polycarbonate bottle[a]	Median	46.1	2.48
	(Range)	(15.3 – 75.6)	(0.82 - 4.07)
Beverage can[b]	Median	0.26	0.01
	(Range)	(1.47 – 46.48)	(0.08 – 2.50)
Thermal paper[c]	Median	13.0	0.62
	(Range)	(NA – 49.68)	(NA – 2.38)

Range: minimum – maximum; EIA via PK: estimation via two-compartment pharmacokinetic model; EIA via F_{ue}: estimation via simple fraction of urinary excretion (F_{ue}).

[a] Two times ingested 200mL water in polycarbonate bottle.

[b] Two times ingested 200mL water in beverage can.

[c] One time touched thermal paper for 15minutes (three sheets of thermal paper (9 X 15 cm^2 each)).

Ⅳ. Discussion

4.1. Comparison of time profiles of urinary BPA for first 6 hours after exposure

The present study determined differences in the levels of BPA in urine samples from seven volunteers exposed to BPA from usual consumer products such as polycarbonated container, canned drink and thermal receipt in specified design. Cumulative urinary amounts of BPA until 6 hours after the exposure were about 1.1 to 4.8 times higher in the exposed relative to the control group (Table 3), and they were significantly different among the exposed to polycarbonate cup users and the corresponding controls ($p < 0.05$). Although the GM ratio of the exposed to the control appeared indistinguishable for canned drink and thermal receipt, overall range of levels of BPA in the urine were higher in the exposed and GM from the limited number of the subjects did not allow the separation. GMs of all collection (n = 6 per each subject) of urinary BPA amounts until 6 hours showed noticeably higher relative to those of the controls – 0.33 vs 0.13 for polycarbonates ($p < 0.05$), 0.24 vs 0.08 for canned drink ($p < 0.05$), 0.18 vs 0.09 for thermal receipt ($p < 0.1$).

Considering other various sources of BPA in daily life and its relative short

biological half-time (3.42 hours, Fig. 2), single exposure to sources of BPA of interest seemed not a big contributor the its body burden. When we compare the urinary levels from control and exposed 18 hours more after 6-hr exposure experiment, the urinary BPA did not distinguishable between the two groups; moreover, since our participants went back their normal life and ate different dinner, the control also had high levels after eating their general dinner (data not shown). For those reasons, sophisticated designed exposure onto humans were need to measure the actual contribution to body burden from the popular consumer products of BPA.

The daily profiles of BPA amounts in urine from the daily monitoring even including after the confined exposure in the present study was similar to Teeguarden et al., 2011, where the subjects were provided with diet with canned foods and juices to represent a potentially high BPA dietary exposure scenario. Based on the urinary elimination data, they reported time course pattern that amounts after having dinner were higher than having other meals. Although our intervention set limits on fasting of breakfast and providing BPA free of lunch, urinary BPA amounts were responded to exposure as time went by the evening.

4.2. Estimation of absorbed amounts of BPA by exposure

Two-compartment model of PK model was utilized to estimate absorbed amount of each exposure even after optimization of Volkel's human exposure experiment with oral administration of isotope-labelled BPA (Volkel et al., 2002). The PK model successfully explain the disposition of urinary BPA in the present study. Time profile of urinary BPA amounts in our study was reflected this; concentration of blood rapidly increased within 5 minutes after exposure, reached C_{max} in 30 minutes after exposure. In spite of the quick absorption, excreted BPA was about 10% of dose in 30 minutes after exposure, 70% in 6 hours, 9% percent in 12 hours and 1% in a day according the model; probably it is the reason why it is difficult to detect precise contribution from the source of interest in multiple exposure in usual daily life. In the present study, we monitored cumulative amounts of BPA in urine since it represented about 70 percent of dose and it could be applied or compared to other human studies which could be easily confounded with other candidate source of BPA in monitoring time over 6 hours. More interestingly, our second exposure after 6^{th} hour (4 hours for thermal receipt) did not reflected well to the urinary BPA until 8^{th} hours. Because of limitation associated with inconvenience of the volunteers, we could not confined them in the experimental place after 8 hours; and that is

the reason why we only focused the first exposure for the first six hours. Compared to the second exposure, first exposure was not affected with other candidate confounder such as diet. Our subjects were asked skipping breakfast on experiment day. Even lunch that we provided was commercially purchased, possible contamination could not be excluded.

In results of F_{ue} model, Callan et al., 2013 reported the median daily intake calculated for 26 pregnant women during their third trimester was 0.06 μg/kg/day (range 0.01 – 0.14 μg/kg/day), and the median BPA concentrations was 2.41 μg/L (range <LOD – 5.66 μg/L). This estimated intake is lower than that reported for pregnant women in the MoBa study, but similar to the calculated intakes for pregnant women from NHANES in the United States and the Generation R study in the Netherlands (Ye et al., 2009). After all, the estimated daily intake (EDI) using excretion rate equation was highly lower than tolerable daily intake (50 μg/kg/day).

4.3. Relative contribution of internal dose of BPA by exposure

There were several research that dietary intervention and intervention of major one of BPA exposure sources: polycarbonate plastics and epoxy resin cans; however, to our knowledge, the present study is the first to try intervention experiments that one participant was exposed to major three sources of human BPA exposure and collecting repetition urines for 24 hours. Thus, this study tried to figure out contribution of the impact on human by major exposure sources.

Other studies compared between during intervention and pre or post intervention, but our study compared between exposed and control group. We obtained results that statistically difference was observed to comparison between exposed and control group for exposing polycarbonate bottle and beverage can. Carwile et al., 2009 demonstrated that the corresponding increase in urinary BPA concentrations after use of polycarbonate drinking bottles. In that study, urinary BPA concentrations were higher when participants consumed the majority of cold beverages from polycarbonate bottles during a week intervention compared with a washout phase in which polycarbonate bottles were avoided. Also, in Rudel's study, urine levels of BPA decreased

significantly during the fresh foods intervention, suggesting that most BPA intake came from food packaging or meals outside the home. The study of Bae and Hong (2015) demonstrated that consuming canned beverage significantly increased the urinary BPA concentration compared with consuming the same beverage in the glass bottle. Difference of group in thermal paper exposure experiments did not observe, because diet is a significant source of exposure for BPA (Sathyanarayana et al., 2013).

The comparison results of urinary BPA amounts suggested that those results could be reflected as BPA concentration of three exposure sources. Urinary BPA amounts and concentrations of sources for polycarbonate bottles, both levels were higher than levels of other exposure events, and both levels about beverage can also were detected in the next order. Makris et al., 2013 reported that water consumption from polycarbonate bottles was significantly associated with females. Thus, our study represented that BPA exposure events contributed to urinary BPA amounts.

In addition, BPA concentrations were measured by sources of three exposure events and rice porridge as lunch in this study. BPA was found in all exposure sources but BPA concentrations of food (n = 36) were mostly below the limit of detection of 0.69 ng/g. Polycarbonate bottles and beverage cans were subjected to actual simulated use by temperature. BPA was detected in 200mL water samples in polycarbonate bottles (n = 6, geometric mean; GM = 0.0261 ng/mL) and tin canisters (n = 6, GM = 0.0079 ng/mL) at room temperature,

above the LOD of 0.0007 ng/mL (data not shown). At 50 ℃, BPA was detected in 200mL water samples in polycarbonate bottles (n = 6, GM = 0.0328 ng/mL) and tin canisters (n = 6, GM = 0.0106 ng/mL). At 100 ℃, BPA was detected in 200mL water samples in polycarbonate bottles (n = 6, GM = 0.1764 ng/mL) and tin canisters (n = 6, GM = 0.1389 ng/mL). Also BPA concentrations of thermal receipt paper (n=12) were performed migration test, not simulating actual use as skin absorption, were ranged from 0.005 to 9.63 mg/g (GM = 0.24; median = 3.74).

BPA concentrations were measured at polycarbonate bottle and beverage can, simulated actual use by different temperature. It was simulated that putting water in the container during 30 minutes before drinking like actual use. As used temperature increased, so did detect BPA concentrations in containers. In other study, BPA was found to be released within 24 hours from four brands of baby bottles at room temperature (24℃), 40℃ and 100℃, increased temperature led to higher release of BPA from the baby bottles (Li et al., 2010). In Brede et al., 2003's study, bottles subjected to dishwashing, boiling and brushing exhibited a significant increase in the migration levels, and migration levels rose on repeated use. BPA levels of these previous studies had similar to our BPA concentrations in polycarbonate bottle. Although researcher focused on real use of container, beverage canisters might be had unknown distribution processes. There was an effect of storage time on BPA migration from pepper

cans (Munguia-Lopez and Soto-Valdez. 2001). Therefore, detected BPA concentrations might be induced underestimate of impact of long storage time of beverage cans. Although it was difficult that direct comparison with BPA concentrations of other sources, thermal paper detected great BPA concentrations in migration test, not normal use simulation; however, our results had similar to other levels of BPA concentrations (Liao and Kannan. 2011).

Ⅴ. Conclusions

The present study demonstrated that BPA exposure contributed to urinary BPA amounts and showed the extent of contribution of each three exposure. In intervention, polycarbonate bottles exposure was greater contributions because of significant differences (p = 0.029) of cumulative urinary BPA amounts between exposed and control group. In general life, dietary factor (especially dinner) had a relatively large portion for exposure to BPA, we apprehended the exposure properties in urinary BPA during 24 hours. Two approaches were performed that estimated intake amounts using two-compartment pharmacokinetic model and F_{ue} model. Estimated intake using two-compartment pharmacokinetic model was higher than using F_{ue} model. Researchers considered that pk model was reflected more accurately actual pharmacokinetic action, suggest that F_{ue} model is needed to reset.

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국문초록

노출 중재 동안 소변 중 비스페놀 A의 프로파일 연구

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비스페놀 A (BPA)는 폴리카보네이트 소재 플라스틱이나 에폭시 수지, 감열지 등의 제품에 사용되는 화학물질이다. 내분비계 장애물질로 분류되며, 체내에서 에스트로겐과 유사한 효과로 인해 호르몬 수용체에 교란을 일으켜서 부정적인 건강 영향을 야기한다. 인간의 생활과 밀접하면서 쓰임이 다양한 제품에 존재하며, 다양한 경로로 노출될 수 있기 때문에, 그 심각성은 더해 가고 있다. 따라서, 본 연구에서는 1) BPA의 세 가지 주요 노출원별 기여평가를 하고, 2) 소변 데이터를 활용해 노출량을 추정하여 BPA의 노출 특성을 파악하

고자 한다.

성인 남성 7명을 노출군과 비노출군으로 나눠, 일주일 간격으로 3일, 8시간 동안 중재를 하였다. 첫째 날, 노출군은 폴리카보네이트 용기에 음료를, 둘째 날, 캔음료를 섭취하게 하였고, 셋째 날, 감열지를 만지게 하였다. 8시간 이후로 각 개인은 일상 생활을 하였고, 24시간 동안의 모든 소변을 수집하고, 분석하였다. BPA는 최초 노출 이후, 6시간 후에 노출된 양의 70%가 배설되고, 반감기가 3.42시간인 점을 고려하여, 두 번째 노출이 있기 전 첫 번째 노출 기간인 6, 4 시간 모니터링의 데이터로 분석을 실시하였다.

첫 노출 동안, 폴리카보네이트 용기에 의한 노출에서만 노출군과 비노출군의 차이가 통계적으로 유의했다 (p = 0.029). 나머지 노출원에서의 노출군, 비노출군의 차이와, 노출군에서 세 노출원의 의한 차이는 통계적으로 유의하지 않았지만, 노출군과 비노출군의 소변 중 축적 BPA 양은, 폴리카보네이트 용기에 의한 노출에서 3.6배 (2.75 vs. 0.77 μg), 캔음료에 의한 노출에서 1.4배 (1.37 vs. 0.99 μg), 감열지에 의한 노출에서 1.9배 (0.86 vs. 0.45μg)의 차이가 있었다. 또한, 소변 중 BPA 양을 바탕으로 노출원별 섭취량을 추정한 결과, 폴리카보네이트 용기 노출은 약 0.7 ~ 2.6 μg, 캔음료 노출은 약 0.3 ~ 2.1 μg, 감열지 노출은 약 0.1 ~ 1.5 μg 이었다.

이러한 결과를 바탕으로, 세 가지 주요 노출원의 기여의 정도를 알 수 있었고, 중재를 하는 동안, 폴리카보네이트 용기에 의한 노출이 다른 노출원보다 소변 중 축적 BPA 양에 기여도가 컸으며, 일상 생활을 하는 중에는, 특히 식이를 통한 노출이 소변이라는 바이오마커로 반영이 된다는 것과 하루 동안의 BPA 노출패턴을 파악할 수 있었다. 노출 실험 동안 BPA의 다른 노출이 없도록 제한을 했음에도 불구하고 소변 중 BPA의 개인내 변동이 컸다는 것은 다양한 환경노출과 개인 pk 를 고려한 변이에 대한 추가 연구가 필요하다는 것을 의미한다. 또한, 본 연구에서의 F_{ue} 모델은 외적 노출량 추정 시 일반적으로 사용하는 모델이지만, 소변 배설 분율의 값이 1로써, 실제 체내 약동학적 메커니즘이 반영된 two-compartment pk model 의 노출량-변환 차트를 이용한 추정 섭취량보다 underestimate 되었다고 본다. 따라서, F_{ue} 값의 재설정을 제시함으로써, 정교한 노출평가를 할 수 있는 기반을 마련했다고 할 수 있겠다.

주요어: 비스페놀 A, 노출원, 제품, 중재 실험, 타임프로파일, 추정 섭취량, 약물 동태학

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