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Pharmacokinetics of bisphenol S in humans after a single oral administration

경구 투여에 따른 비스페놀 S 의 인체 내 약물동태학 연구

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Abstract

Pharmacokinetics of bisphenol S in humans after a single oral administration

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Bisphenol S (BPS) has been introduced as a substitute for bisphenol A (BPA), and widely used in the manufacture of polycarbonate plastics, epoxy resins and thermal papers. In spite of its adverse health outcomes and widespread exposure, pharmacokinetic data for BPS are not available for either animals or humans. The objective of the study is to describe pharmacokinetic characteristics of BPS in human body after a single oral administration with a compartmental pharmacokinetic model. Seven healthy young adults were orally exposed to 8.75 μg/kg bw of deuterated BPS (d_4-BPS), and serum and urine samples were collected for 48 hours. The concentrations of total and unconjd d_4-BPS in

samples were measured using HPLC-MS/MS. Based on the time-concentration profiles in serum and urine, non-compartmental analysis was performed, and two-compartment model was constructed and validated. As a result of non-compartmental analysis, total d_4-BPS was rapidly absorbed within one hour (0.7 ± 0.3 h) after oral administration, and excreted in urine with terminal half-life of less than 7 h (6.8 ± 0.7 h). Fractional urinary excretion (F_{ue}) of total d_4-BPS for 48 hours was 92 ± 17 % (67~104 %) for men and 70 ± 36 % (59~77 %) for women. The two-compartment model well described pharmacokinetic properties of BPS, and its parameter estimates were consistent with those from non-compartmental analysis. Comparing with pharmacokinetic data for BPA from other studies, BPS is retained in human body longer than BPA, although both are mostly excreted from the human body within 24 hours. This study provides information on absorption, distribution, metabolism and elimination of BPS in human body, and the pharmacokinetic model can be utilized for estimating exposure dose of BPS, contributing to more realistic exposure assessment.

Key words: Bisphenol S, oral administration, human pharmacokinetics, ADME, pharmacokinetic model

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I. Introduction

Bisphenol S (BPS) is increasingly used in the manufacture of polycarbonate plastics, epoxy resins in food can lining, baby bottles, and thermal papers (Liao et al., 2012a; Liao and Kannan, 2013; Simoneau et al., 2011). It has been introduced as a substitute for bisphenol A (BPA), one of the representative endocrine disrupting chemicals, due to the structural analogy and higher thermal stability (Lotti et al., 2011). Widespread occurrence of BPS in environment, including sediments, sewage sludge, surface water, and indoor dust has been documented in various countries (Liao et al., 2012b; Liao et al., 2012c; Yamazaki et al., 2015; Yu et al., 2015).

Exposure to BPS can occur through ingestion, inhalation and dermal absorption; however, the primary pathway to the human body is considered to be dietary exposure (Liao et al., 2012a; Liao et al., 2012b). Human biomonitoring data from several studies have demonstrated that exposure of general population to BPS is widespread (Liao et al., 2012d; Yang et al., 2014; Zhou et al., 2014). Furthermore, it was reported that the urinary concentrations as well as the detection frequency of BPS in United States adults had gradually increased from 2000 to 2014, which indicated the increasing trend of human exposure to BPS (Ye et al., 2015).

Limited studies have reported a wide range of adverse health outcomes of BPS, including endocrine disruption, cytotoxicity, mutagenicity, obesity, and reproductive and developmental toxicity. An *in vitro* study demonstrated that BPS showed a weak estrogen activity by binding to human estrogen receptors with lower effectiveness than BPA, and also acted as a weak agonist for human androgen receptors (Molina-Molina et al., 2013). A recent study demonstrated that BPS non-monotonically reduced the basal testosterone secretion in human fetal testes and exhibited anti-androgenic effects (Eladak et al., 2015). It was also documented that exposure to higher concentrations of BPS led to oxidative stress in mouse hepatocytes and renal cells (Zhang et al., 2016), and in rat testis, which could lead to the alteration of reproductive functions in adults (Ullah et al., 2016), and caused mutagenicity by inducing significant DNA damage in HepG2 cells only after 24 h of exposure (Fic et al., 2013). An *in vivo* study reported that BPS could be an obesogen at low doses and after perinatal and chronic exposure in male mice (Moral et al., 2016). These data were substantiated by increase in lipid accumulation and differentiation in primary human preadipocytes (Boucher et al., 2016).

The metabolism of BPS was investigated in several *in vitro* studies, while no *in vivo* study was reported (Skledar and Mašič, 2016). The scheme of metabolic pathways of BPS is described in Fig. 1. Following ingestion, the majority of BPS was metabolized into BPS-glucuronide mainly in the liver by the hepatic

enzyme UGT1A9, although a low portion of BPS could be previously metabolized in the intestine by UGT1A10 (Skeldar et al., 2015; Skeldar et al., 2016). An *in vitro* study demonstrated that 10.5% of BPS was metabolized into BPS-sulfate in HepaRG, one of the human hepatic cell lines with high biotransformation capabilities, while 85.8% was transformed into BPS-glucuronide (Le Fol et al., 2015). The *ortho* hydroxylated BPS was also formed in the oxidative pathway primarily by CYP450 as a minor BPS metabolism (Skeldar et al., 2016).

Human pharmacokinetic data derived from controlled dosing study provides information on how human body responds to environmental chemicals. Since general populations have been increasingly exposed to BPS, it is essential to understand how it is absorbed, distributed, metabolized and excreted in human body. To our knowledge, pharmacokinetic data of BPS are not available in either animals or humans, while several pharmacokinetic and physiologically based pharmacokinetic (PBPK) models for BPA are developed (Völkel et al., 2002; Kawamoto et al., 2007; Doerge et al., 2011; Fisher et al., 2011). Therefore, the objective of the study is to describe pharmacokinetic characteristics of BPS in human body after a single oral administration with a compartmental pharmacokinetic model.

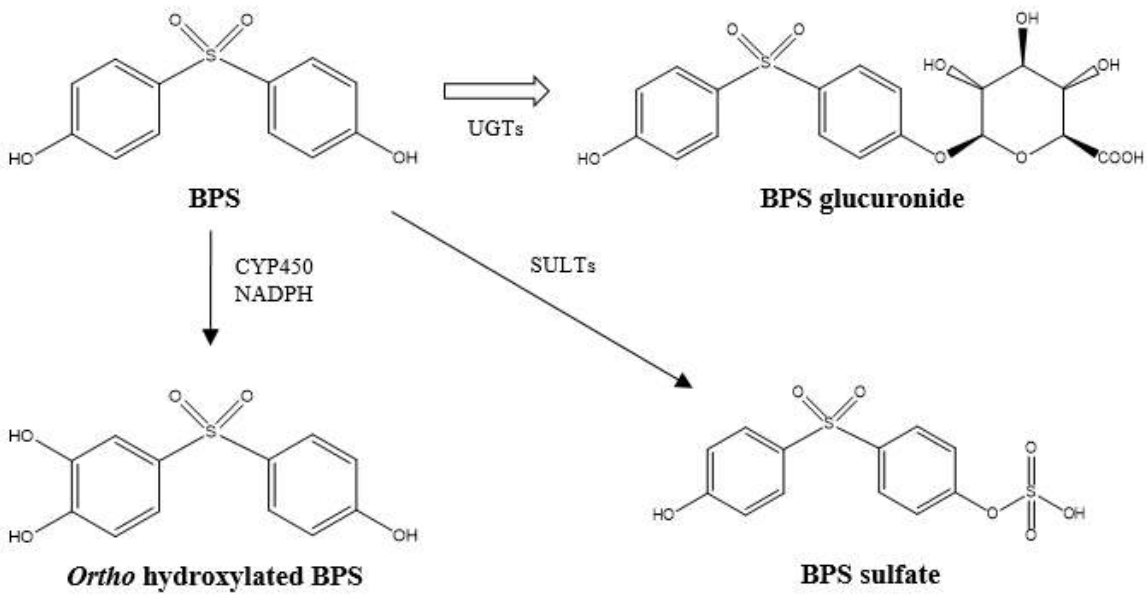

Fig. 1. Scheme of metabolic pathways of bisphenol S. UGT, uridine 5'-diphospho-glucuronosyltransferase; CYP450, cytochrome P450 enzyme; NADPH, nicotinamideadenine dinucleotide phosphate; SULT, sulfotransferase [adapted from Skledar and Mašič (2016)].

II. Materials and Methods

1. Chemicals and Reagents

d_4-bisphenol S and $^{13}C_{12}$-bisphenol S were purchased from Toronto Research Chemicals, Inc. (Toronto, ON, Canada). Ammonium acetate (≥97%), β-glucuronidase (130,052 units/mL, Helix pomatia, HP-2, containing 1,036 units/mL of sulfatase), and absolute ethanol (USP grade) were purchased from Sigma-Aldrich Laboratories, Inc. (St. Louis, MO, U.S.A.). Ethyl acetate, water, and methanol (HPLC grade) were purchased from Avantor (Center Velly, PA, U.S.A.).

2. Study Design and Sample Collection

A dose of 8.75 μg/kg bw of d4-BPS was orally administered in a chocolate cookie to four healthy male and three healthy female volunteers, who were recruited from public notice of the present study in August, 2016. The administered dose was determined based on specific migration limit (SML) of bisphenol S (0.05 mg/kg food) established by European Union, and daily food intake (0.175 kg food/kg bw/day; 0.1L non-milk beverage/kg bw/day + 0.025kg milk/kg bw/day + 0.05kg food/kg bw/day) (Food and Agriculture Organization of the United Nations and World Health Organization, 2009). Deuterated BPS (d_4-BPS) was used to avoid potential effects of background concentrations of BPS. A dosing solution (10 mg/mL) was prepared by dissolving d_4-BPS in 20% ethanol, and spiked to a chocolate cookie just before dosing. All participants were requested to fast at least 8 h before dosing and to refrain from drinking and smoking one day before and during the experiment period. The study was conducted according to protocols approved by the Institutional Review Board of Seoul National University, Korea (IRB No. 1609/001-004). All participants were informed about the study design, and provided written informed consent in advance of the experiment.

Blood samples were collected just before dosing (0 h) and at time point of 0.25, 0.5, 0.75, 1, 2, 3, 4, 6, 8, 12, 24, 48 h after administration. Blood samples were taken into serum-separating tube using intravenous cannula by a nurse. After mixing thoroughly by repeatedly inverting the tube, samples were allowed to clot at room temperature for 30 min and centrifuged for 10 min at 1300g. Separated serum was transferred to polypropylene microcentrifuge tubes and stored at -80℃ until analysis. Likewise, urine samples were collected just before dosing (0 h) and at time point of 0.5, 1, 2, 3, 4, 6, 8, 12, 24, 48 h after administration. Urine samples were taken into 1 L polypropylene bottle and the volume of each urine sample was also measured. Collected urine was transferred to polypropylene microcentrifuge tubes and stored at -80℃ until analysis.

3. Sample preparation

Analysis of total d_4-BPS in serum and urine was performed by following the method as previously described with some modifications (Asimakopoulos et al., 2014). Briefly, after thawing at room temperature, an aliquot of 500 μL of serum or urine was transferred into a 15 mL polypropylene tube, and 10 μL of $^{13}C_{12}$-BPS (0.25 ng/μL) was spiked as internal standard. The sample was buffered with 340 μL of 1 M ammonium acetate containing 22 units of β-glucuronidase (prepared by spiking 50 μL of β-glucuronidase into 100 mL of 1 M ammonium acetate solution), and digested at 37℃ for 12 h in an incubator. Thereafter, the sample was extracted three times with 3 mL of ethyl acetate each time (3 × 3 mL). For each successive extraction, the mixture was shaken in an oscillator shaker for 60 min, and then centrifuged at 4000 g for 10 min. The supernatants were combined in another polypropylene tube, and concentrated to near-dryness under a gentle nitrogen stream. The extract was reconstituted with 50 μL of methanol, vortex mixed, and transferred into an auto-sampler vial for high performance liquid chromatography tandem-mass spectrometry (HPLC-MS/MS) analysis. Concentrations of unconjugated d_4-BPS in serum and urine were measured using the same methodology described above without the enzymatic hydrolysis step.

4. HPLC-MS/MS analysis

An API 4000 electrospray triple quadrupole mass spectrometer (ESI-MS/MS; AB SCIEX, Framingham, MA, U.S.A), coupled to Nexera HPLC system (Shimadzu, Kyoto, Japan) was used for identification and quantification of d_4-BPS. The chromatographic separation was performed by YMC-Pack ODS-AQ C18 column (150 × 2.0 mm, 3 um; YMC, Kyoto, Japan), and the sample injection volume was 10 μL. The mobile phase was composed of 10 mM ammonium acetate (solvent A) and methanol (solvent B) at a flow rate of 200 μL/min, and the gradient was as follows: 0-2 min, 15% B; 2-9 min, 15-90% B; 9-12 min, 90-99% B; 12-15 min, 99% B; 15-20 min, 15% B. Target analytes were detected in the negative ion multiple reaction monitoring (MRM) mode, and the MRM ion transitions monitored were as follows: 253.0 to 109.9 for d_4-BPS and 261.1 to 113.7 for $^{13}C_{12}$-BPS. The electrospray ionization voltage was kept at -4.5 kV. Nitrogen was used as CAD gas and curtain gas, and the flow rates were set at 5 psi and 20 psi, respectively. The source heater was kept at 400 ℃, and the nebulizer gas (ion source 1) and the heater gas (ion source 2) were set at 40 psi. The compound specific MS/MS parameters are shown in the supplementary information (Table S1).

5. Quality Assurance and Quality Control

Analysis method validation was performed by spiking three different concentrations of d_4-BPS into serum and urine on three different days and evaluating intra- and inter-day accuracy and precision, ranging from 100 to 113% and 6 to 19%RSD for serum and from 90 to 98% and 4 to 16%RSD for urine, respectively. A procedure blank, a spiked blank and two matrix-spiked samples were analyzed for each set of 18 samples, following the same procedures as described above. Procedure blanks were prepared using HPLC grade water instead of serum or urine sample. d_4-BPS was not detected in any of the procedure blanks. Matrix-spiked samples were prepared at the first day of the experiment by spiking known concentrations of d_4-BPS to blank serum or urine. The recoveries of d_4-BPS from matrix-spiked samples ranged from 85 to 124% for serum and 88 to 117% for urine, and the relative standard deviation (RSD) was 9.4 % for serum and 8.6 % for urine. Methanol, a solvent, was injected in the beginning and middle of each sample set in order to check for carry-over of d_4-BPS from sample to sample.

Calibration curve, ranging from 0.04 to 200 ng/mL, was plotted based on logarithmic ratio of the peak area of d_4-BPS to the peak area of the internal standard versus the logarithm of the d_4-BPS concentration (Yoon et al., 2015), whose regression coefficient (R) was >0.999 in serum and urine. The limits of quantitation (LOQ) was determined based on the lowest point where the logarithm form of calibration curve, consisting of very low concentrations, became the straight line, and the LOQ of d_4-BPS was 0.02 ng/mL in both serum and urine.

6. Pharmacokinetic analysis

6.1. Non-compartmental analysis

The time-concentration profiles of total and unconjugated d_4-BPS in serum were analyzed with non-compartmental analysis using WinNonlin (Pharsight, St. Louis, MO, U.S.A.). Several descriptive pharmacokinetic parameters, such as maximal concentration (C_{max}), peak time (T_{max}), area under the curve (AUC) and mean residence time (MRT), were estimated. The fractional urinary excretion (F_{ue}) was calculated based on the amount of total d_4-BPS excreted in urine over 48 h divided by orally administered dose.

6.2. Construction of pharmacokinetic model

Two-compartment model was constructed to describe the pharmacokinetic properties of BPS in human body after a single oral administration (Fig. 2). The rate of absorption, distribution, metabolism, and excretion was assumed to be first-order kinetics. The constructed model was fitted into the time-concentration profile in serum and urine from randomly picked four participants (two males and two females) using Berkeley Madonna (University of California, Berkeley, CA, U.S.A.). The calibrated model was validated by evaluating the model prediction using serum and urine data from the rest of three participants.

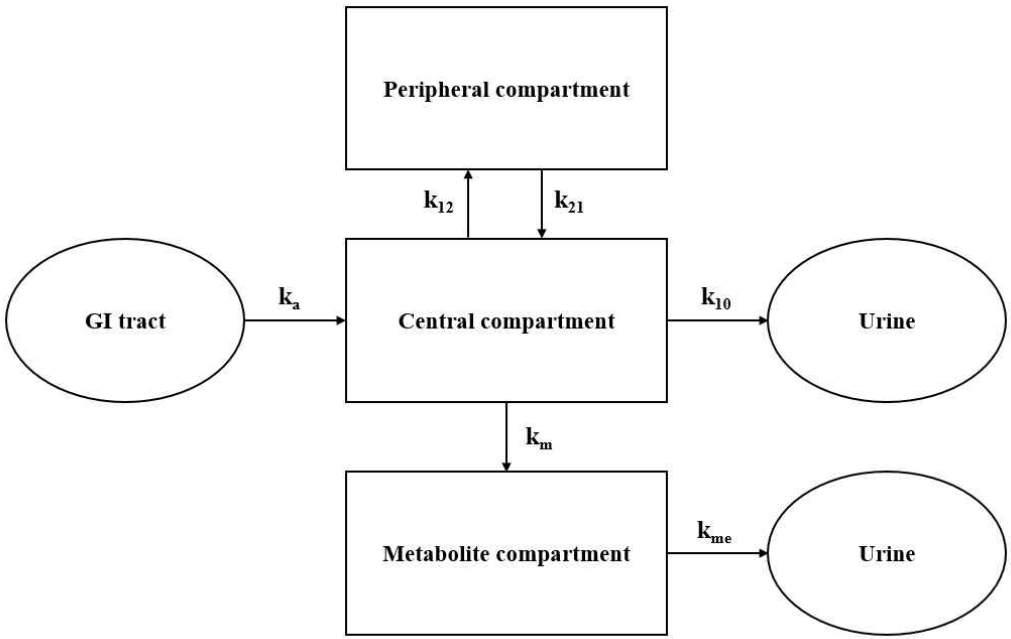

Fig. 2. Structure of two-compartment model for bisphenol S. Abbreviations: k_a – BPS absorption in gastrointestinal tract; k_{12} – BPS disposition from central compartment to peripheral compartment; k_{21} – reabsorption from peripheral compartment to central compartment; k_{10} – unconjugated BPS elimination at central compartment; k_m – conjugated BPS formation; k_{me} – conjugated BPS elimination at metabolite compartment.

III. Results

1. Participant characteristics

Four healthy males and three healthy females participated in the study, and demographic characteristics of participants are shown in Table 1. The average age of the participants was 27.4 (26.8 ± 2.63 for male, 28.3 ± 6.66 for female), and the average BMI was 24.6 (25.3 ± 2.99 for male, 23.7 ± 4.62 for female). Two participants were past smokers, and others were never smokers. Four participants were frequent drinkers (>once a week), one participant was less frequent drinker (> once a month), and one participant was abstainer (<once a year). Two participants were taking medicine for cold and depression during experiment period.

Table 1. Demographic characteristics of participants.

Participants	Gender	Age	Height (cm)	Body weight (kg)	BMI
A	male	29	176	73	24
B	male	23	177	90	29
C	male	28	173	78	26
D	male	27	184	74	22
E	female	36	167	80	29
F	female	24	160	53	21
G	female	25	163	55	21

2. Pharmacokinetic characteristics of BPS

The semilogarithmic plot of time-concentration profile for total and unconjugated d_4-BPS in serum is presented in Fig. 3. After oral administration, the serum concentrations of total and unconjugated d_4-BPS increased rapidly within one hour. After the peak time, total and unconjugated d_4-BPS concentrations decreased in two phases, fast phase and slow phase in order. The rate of decline drastically changed around 6 hours after oral administration. While total d_4-BPS was observed in serum until 48 hours, unconjugated d_4-BPS was not detected at the time point of 48 hours.

Cumulative urinary excretion of total d_4-BPS from seven participants is presented in Fig. 4. The fractional urinary excretion (F_{ue}, %) of total d_4-BPS was an average of 82% (range: 59~104%). The average F_{ue} of total d_4-BPS in males was 92% (range: 67~104%), and that in females was 70% (range: 59~77%). On the other hand, the F_{ue} of unconjugated d_4-BPS was 2.5% on average (range: 0.9~4.1%). The average F_{ue} of unconjugated d_4-BPS in males was 2.6% (range: 1.6~4.1%), and that in females was 2.4% (range: 0.9~3.3%). Cumulative urinary excretion of unconjugated d_4-BPS from each participant is shown in Fig. S1.

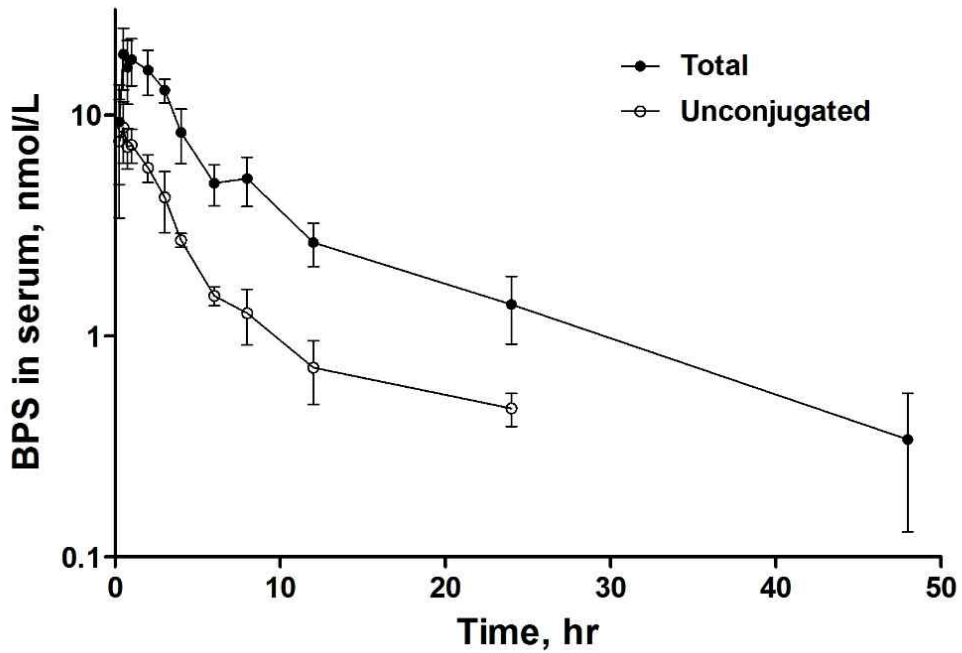

Fig. 3. Time-concentration profile for d_4-BPS in serum. Each point and error bar represent mean and standard deviation of participants (n = 7). Concentrations below LOD were excluded.

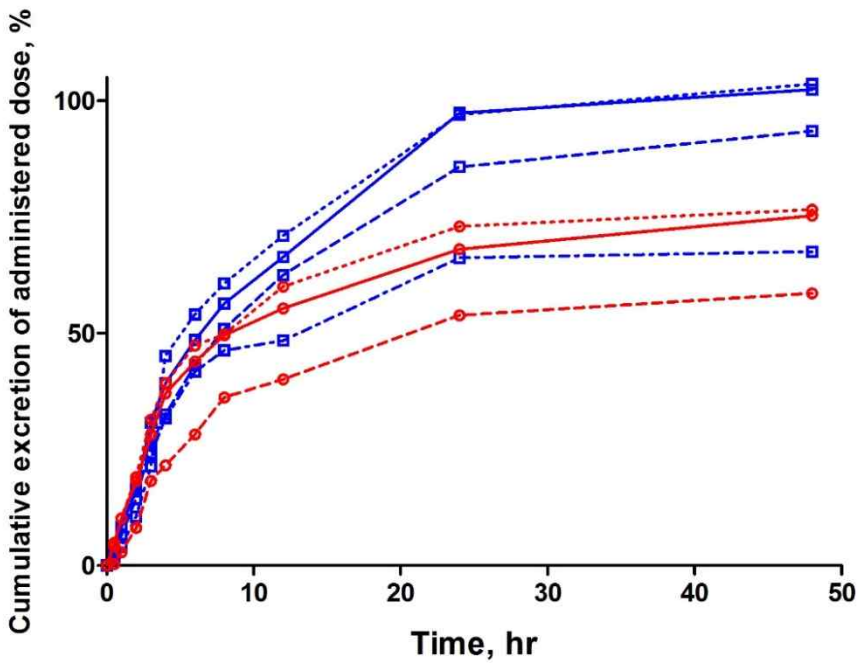

Fig. 4. Urinary excretion of total d4-BPS from seven participants. Cumulative excreted amount of total d_4-BPS at a given time point is expressed as the percentage of administered dose for each participant. Blue square symbols and red circle symbols represent male and female, respectively.

3. Non-compartmental analysis of BPS

Pharmacokinetic parameter estimates for total and unconjugated BPS from non-compartmental analysis are presented in Table 2. The mean T_{max} of unconjugated BPS (0.57 ± 0.31 h) was earlier than total BPS (0.71 ± 0.27 h), though both reached their C_{max} within one hour after oral administration. The mean C_{max} and the mean AUC_{last} of total BPS were 2 and 3.5 times higher than those of unconjugated BPS, respectively. On the other hand, the MRT_{last} of total BPS (9.80 ± 1.03 h) was 1.7 times longer than unconjugated BPS (5.85 ± 0.54 h). The terminal half-life ($T_{1/2}$) was estimated by dividing MRT_{last} by 1.44 (Boroujerdi, 2001), thus the $T_{1/2}$ of total BPS (6.81 ± 0.72 h) was also 1.7 times longer than unconjugated BPS (4.06 ± 0.38 h). Total body clearance was estimated using an equation, $(CL_t)_{oral} = F \times D / AUC$, where F is bioavailability and D is administered dose. Bioavailability, the fraction of an administered dose that reaches the systemic circulation, was assumed as one. Accordingly, the total body clearance of BPS was 17.8 L/h. The parameter estimates for total and unconjugated BPS from non-compartmental analysis by individual and sex are presented in Table S2 and S3.

Table 2. Parameter estimates for total and unconjugated BPS from non-compartmental analysis (n = 7).

Parameter	Unconjugated BPS	Total BPS
T_{max} (h)	0.57 ± 0.31	0.71 ± 0.27
C_{max} (nM)	10.1 ± 2.14	20.5 ± 4.15
AUC_{last} (nM × h)	39.8 ± 2.72	139 ± 22.6
$AUMC_{last}$ (nM × h^2)	233 ± 32.3	1372 ± 330
MRT_{last} (h)	5.85 ± 0.54	9.80 ± 1.03
$T_{1/2}$ (h)	4.06 ± 0.38	6.81 ± 0.72

Each value is presented as mean ± standard deviation of participants. T_{max}, peak time; C_{max}, maximal concentration; AUC_{last}, area under the time-concentration curve in serum between 0 and last observation; $AUMC_{last}$, area under first-moment curve; MRT_{last}, mean residence time, $T_{1/2}$, terminal half-life.

4. Pharmacokinetic modeling of BPS

The two-compartment model for BPS was fitted to time-concentration data in serum and urine from randomly picked four participants, two males and two females, in order to avoid potential effects of sex (Fig. 5). Pharmacokinetic parameters were manually adjusted to arrive at a visual best-fit for the data, and final parameter estimates are provided in Table 3. Half-life of elimination for BPS, which is estimated by dividing 0.693 by k_{10}, is 6.93 h (Boroujerdi, 2001).

The calibrated two-compartment model was validated using time-concentration data in serum and urine from three other participants, two males and one female (Fig. 6). The concentrations of total, conjugated, and unconjugated BPS in serum and the cumulative amount of BPS in urine were well described by the predicted values from the model.

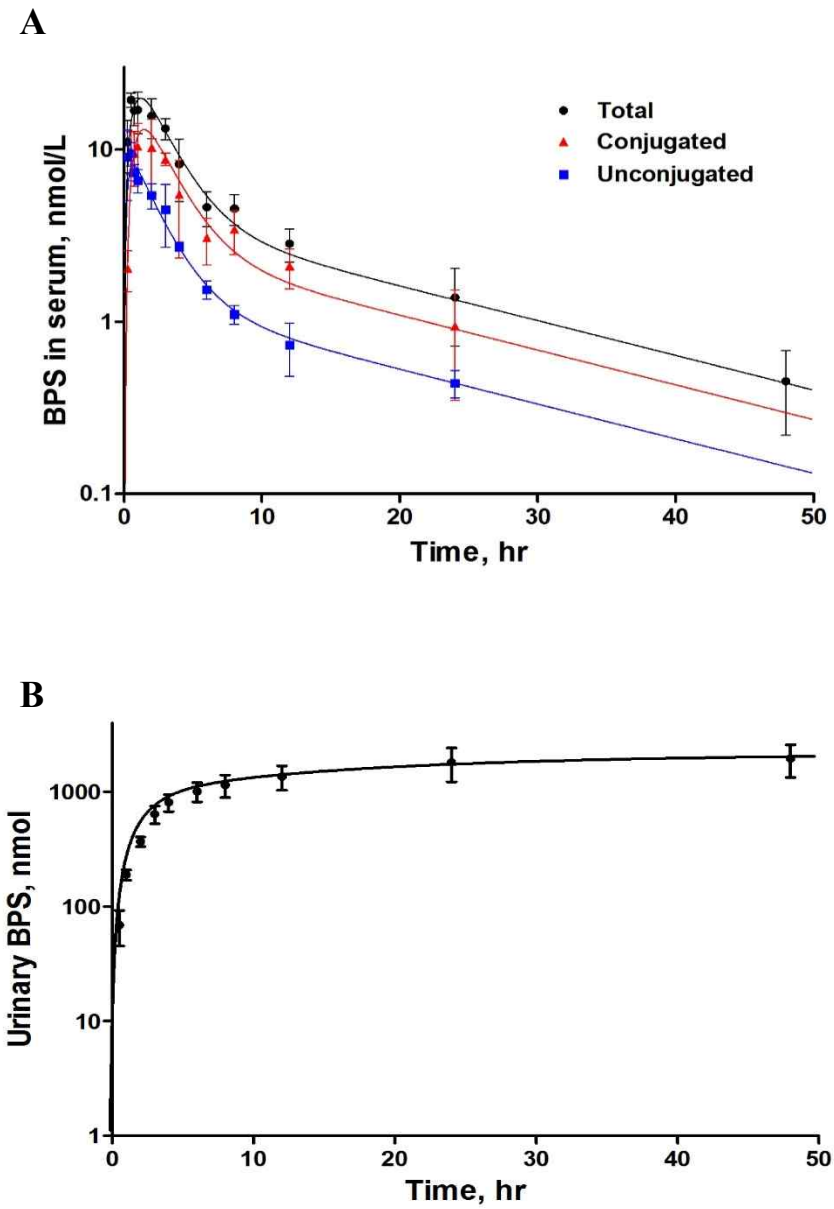

Fig. 5. Model calibration in serum (A) and urine (B) (n = 4). Each point and error bar represent mean and standard deviation of randomly picked four participants. Solid line represents two-compartment model fit to the data. Concentrations below LOD were excluded.

Table 3. Pharmacokinetic parameter estimates from two-compartment model.

Parameter	Notes	Value
k_a, /h	GI tract → central compartment	4.20
k_{12}, /h	central compartment → peripheral compartment	0.20
k_{21}, /h	peripheral compartment → central compartment	0.10
k_{10}, /h	BPS elimination	0.10
k_m, /h	BPS → BPS metabolites formation	0.12
k_{me}, /h	BPS metabolites elimination	1.75
V_{dc}, L	volume of distribution of central compartment	205
V_{dm}, L	volume of distribution of metabolite compartment	7
$T_{1/2}$, h	half-life of elimination for BPS	6.93

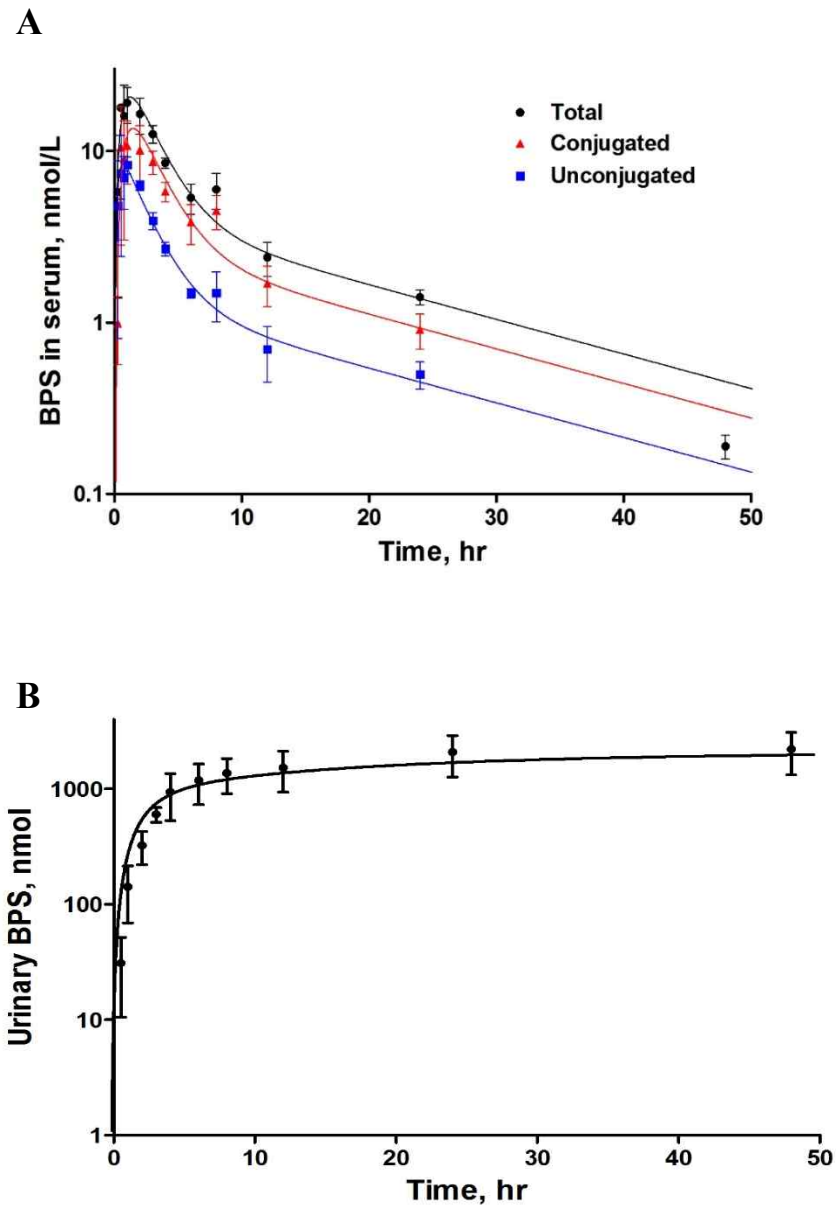

Fig. 6. Model validation in serum (A) and urine (B) (n = 3). Each point and error bar represent mean and standard deviation of three participants other than four people for model calibration. Solid line represents two-compartment model fit to the data. Concentrations below LOD were excluded.

IV. Discussion

Despite its various adverse health outcomes and widespread use as a substitute for BPA, pharmacokinetic data or model for BPS were not available, while several studies had reported pharmacokinetic data or model for BPA in animals and humans. Therefore, the present study investigated the pharmacokinetic characteristics of BPS in humans after a single oral administration of a dose of 8.75 μg/kg bw, and established a two-compartment model to describe the movement of BPS.

The semilogarithmic plot of time-concentration profile in serum (Fig. 3) demonstrated that the pharmacokinetics of BPS could be explained by a two-compartment model. The volume of distribution for central compartment (V_{dc}) was 205 L and that for metabolite compartment (V_{dm}) was 7 L, and all the rate constants in the model were assumed to follow first-order kinetics. According to the best-fit model, BPS was rapidly absorbed by the gastrointestinal tract within one hour (k_a=4.2) after the oral intake. Unconjugated BPS in central compartment was distributed to peripheral compartment (k_{12}=0.2), and redistributed to central compartment (k_{21}=0.1). Unconjugated BPS was not detected in serum at the last time point, which indicated that it was eliminated in urine within 48 hours (k_{10}=0.1). The half-life of elimination for BPS,

representing the collective effects of metabolism and excretion, was 6.93 h, which was consistent with terminal half-life obtained from non-compartmental analysis. Unconjugated BPS was also metabolized into BPS metabolites (k_m=0.12), primarily into BPS-glucuronide, which were rapidly excreted in urine due to its high water solubility (k_{me}=1.75).

Cumulative urinary excretion of total BPS in Fig. 4 demonstrated that an average of 82% of administered BPS was excreted in urine within 48 hours. The average fractional urinary excretion of total BPS for male (92%; range: 67~104%) was higher than that for female (72%; range: 59~77%), which indicated that total BPS was eliminated through urine more easily in male than in female, although the difference was not statistically significant (p = 0.16). On the other hand, the average fractional urinary excretion of unconjugated BPS for males and females was similar, which indicated that there was little discrepancy in urinary excretion of unconjugated BPS by sex (Fig. S1). In this study, however, a β-glucuronidase solution containing a small amount of sulfatase was used for enzymatic hydrolysis, suggesting that the amount of BPS sulfate, a minor BPS metabolite, might be underestimated. Therefore, the fractional urinary excretion of total BPS would be higher if sufficient sulfatase was added in sample preparation.

Comparison of pharmacokinetic parameters from non-compartmental analysis between total BPS and total BPA was presented in Table 4. Non-compartmental analysis for BPA was performed based on publically available pharmacokinetic data from Thayer et al. (2015). Both total BPS and total BPA reached their C_{max} within about one hour, though T_{max} of total BPS was slightly earlier than total BPA ($p = 0.14$). The average MRT_{last} and $T_{1/2}$ of total BPS were 2.7 times longer than total BPA, which was statistically significant ($p = 0.0003$). Thus, it is highly probable that BPS was retained in human body longer than BPA, although both were mostly excreted from the human body within 24 hours. On the other hand, the average fractional urinary excretion of total BPS (82%; range: 59~104%) was lower than total BPA (95%; range: 83~109%) during 48 hours, though the difference was not statistically significant ($p = 0.15$).

Pharmacokinetic parameters obtained from the two-compartment model for BPS were compared to those from two-compartment model for BPA from Völkel et al. (2002). According to each model, absorption rate constant (k_a) of BPS was 4.2, and that of BPA was 3.8. The macro rate constants in the model for BPS were 0.28 and 0.05 /h for α and β, respectively, while those for BPA were 0.78 and 0.21 /h, where α was corresponding to all physical and physiologic processes in the distributive phase, and β to distribution and elimination processes, (Boroujerdi, 2001). These results suggested slightly faster absorption and slower elimination of BPS relative to BPA.

Table 4. Comparison of pharmacokinetic parameters from non-compartmental analysis between total BPS (n = 7) and total BPA (n = 14).

Parameter	Total BPS (This study)	Total BPA (Thayer et al., 2015)
Dose (nmol)	2469 ± 455	35120 ± 6791
T_{max} (h)	0.71 ± 0.27	1.08 ± 0.49
C_{max} (nM)	20.5 ± 4.15	1684 ± 474
AUC_{last} (nM × h)	139 ± 22.6	4287 ± 990
$AUMC_{last}$ (nM × h^2)	1372 ± 330	18858 ± 8724
MRT_{last} (h)	9.80 ± 1.03	4.32 ± 1.07
$T_{1/2}$ (h)	6.81 ± 0.72	3.00 ± 0.75

Each value is presented as mean ± standard deviation of participants. T_{max}, peak time; C_{max}, maximal concentration; AUC_{last}, area under the time-concentration curve in serum between 0 and last observation; $AUMC_{last}$, area under first-moment curve; MRT_{last}, mean residence time, $T_{1/2}$, terminal half-life.

Another remarkable discrepancy in pharmacokinetics between BPS and BPA was the proportion of metabolites to the parent compound. The fraction of AUCs for BPS metabolites over total BPS in serum was 70.7 ± 5.51%, while that of BPA was 99.4 ± 0.16% (Thayer et al., 2015). These results indicated that BPS has far lower proportion of metabolites to the parent compound in serum than BPA. Also, the proportion of the excreted amounts of BPS metabolites to total BPS in urine (96.9 ± 1.40%) was smaller than BPA (99.9 ± 0.19%) (Thayer et al., 2015). Suggestively, BPA could be more easily and rapidly metabolized than BPS after oral administration. Considering that the unconjugated form of toxicants is usually more active in toxicological perspective, BPS might pose more risk to human body when a person is exposed to similar levels of BPS and BPA.

According to the parameter estimates of the best-fit model, the volume of distribution for the central compartment (V_{dc}) of unconjugated BPS was relatively large. During the model fit-process, we set its initial value at 37 L (Völkel et al., 2002), same as in BPA due to their similarity of chemical structure; however, the final value of V_{dc} for BPS was 205 L. Although Völkel's V_d value of BPA was about total BPA, the present V_{dc} of BPS was about unconjugated BPS. Therefore, direct comparison might not be appropriate. Also, large V_{dc} might be attributed to distribution of unconjugated BPS to peripheral compartment. Unfortunately, we did not measure the analytes in other tissues

than serum to confirm this speculation. We would like to stress out that the estimate of volume of distribution was empirical value to fit the model and further studies are needed to address this issue.

One of the limitations of the study is that the pharmacokinetic model for BPS is constructed based on a small population of healthy young adults. Furthermore, as other pharmacokinetic data for BPS from controlled dosing study are not available, the model validation was performed using the data from the same study. Thus, additional model validation processes using data from different populations are needed in order to verify universal applicability of the model. Another limitation of the study is that BPS metabolites other than BPS-glucuronide are not considered. Although several *in vitro* studies have demonstrated that most of ingested BPS is metabolized into BPS-glucuronide, a minority of BPS can be still biotransformed into other metabolites, including BPS-sulfate and *ortho* hydroxylated BPS (Le Fol et al., 2015; Skeldar et al., 2016). Therefore, further studies additionally considering minor BPS metabolites are needed to better address pharmacokinetic properties of BPS in human body.

Nevertheless, this study is worthwhile in terms of being based on a human population. Human pharmacokinetic data derived from this study provide more direct information on absorption, distribution, metabolism and excretion of BPS compared to animal data, since humans and animals have discrepant

pharmacokinetic properties, which has been already proved by pharmacokinetic studies of BPA (Völkel et al., 2002). Furthermore, the classic compartmental model for BPS established in the study is simple and practical for dose reconstruction to estimate daily intake dose of general population using human biomonitoring data. Application of human pharmacokinetic model in estimating exposure dose might reduce uncertainties in exposure assessment, including route-to-route, high-to-low dose and intra-species extrapolation (Chen and Blancato, 1987).

V. Conclusion

In conclusion, the present study investigated the time-concentration profiles of BPS in human serum and urine after a single oral administration, and estimated pharmacokinetic parameters through non-compartmental analysis. Furthermore, it was found that the two-compartment model constructed based on the profiles well described the pharmacokinetic characteristics of BPS. This study provides pharmacokinetic data of BPS, which help to understand the absorption, distribution, metabolism and excretion of BPS in human body after oral administration. Moreover, despite the need for further validation process, the human pharmacokinetic model for BPS can be a useful tool for reconstructing exposure dose of BPS by applying human biomonitoring data, ultimately contributing to more realistic exposure assessment.

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VII. Supplementary material

Pharmacokinetics of bisphenol S in humans after a single oral administration

Fig. S1. Urinary excretion of unconjugated d_4-BPS from seven participants.

Table S1. Compound specific MS/MS parameters for determination of bisphenol S in serum and urine.

Table S2. Parameter estimates for total BPS from non-compartmental analysis by individual and sex.

Table S3. Parameter estimates for unconjugated BPS from non-compartmental analysis by individual and sex.

Table S4. Comparison of pharmacokinetic parameters from non-compartmental analysis between unconjugated BPS and unconjugated BPA.

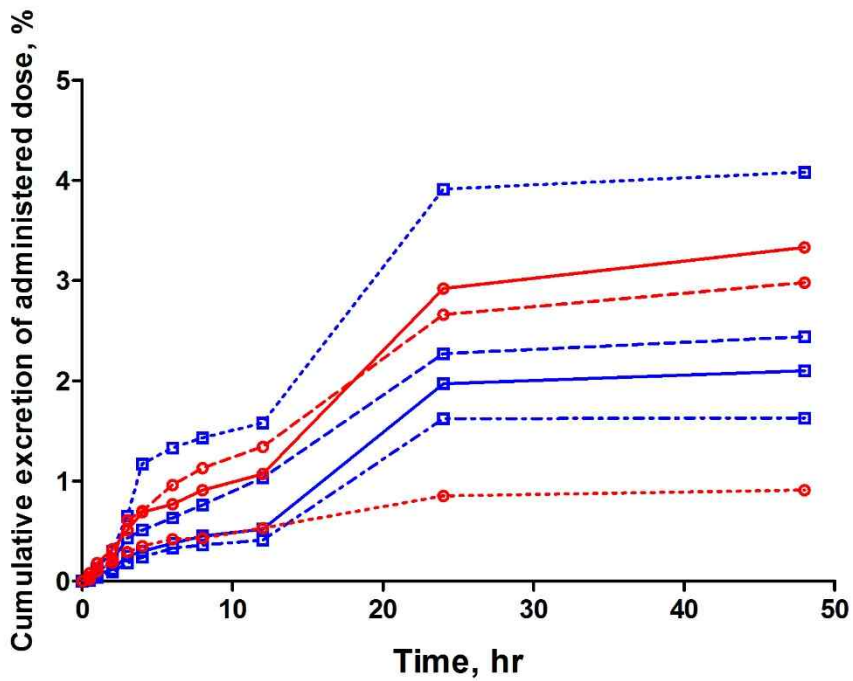

Fig. S1. Urinary excretion of unconjugated d_4-BPS from seven participants. Cumulative excreted amount of unconjugated d_4-BPS at a given time point is expressed as the percentage of administered dose for each participant. Blue square symbols and red circle symbols represent male and female, respectively.

Table S1. Compound specific MS/MS parameters for determination of bisphenol S in serum and urine.

Chemical	RT (min)	Precursor ion	Product ion	DP (V)	EP (V)	CE (V)	CXP (V)
d_4-BPS	8.9	253.0	109.9	-70	-10	-38	-7
$^{13}C_{12}$-BPS	8.9	261.1	113.7	-70	-10	-36	-7

RT, retention time; DP, declustering potential; EP, entrance potential; CE, collision energy; CXP, collision cell exit potential

Table S2. Parameter estimates for total BPS from non-compartmental analysis by individual and sex.

Parameter	A	B	C	D	E	F	G	Male (n = 4)	Female (n = 3)
Dose (nmol)	2498	3101	2667	2550	2732	1827	1906	2704 ± 274	2155 ± 501
T_{max} (h)	1.00	1.00	0.50	0.50	1.00	0.50	0.50	0.75 ± 0.29	0.67 ± 0.29
C_{max} (nM)	22.3	14.4	26.8	20.4	23.3	16.8	19.4	21.0 ± 5.17	19.8 ± 3.26
AUC_{last} (nM × h)	157	124	145	172	149	112	116	149 ± 22.6	126 ± 20.1
$AUMC_{last}$ (nM × h^2)	1421	1208	1301	2068	1378	1110	1118	1500 ± 389	1202 ± 152
MRT_{last} (h)	9.03	9.77	9.01	12.00	9.26	9.91	9.62	9.95 ± 1.41	9.60 ± 0.32
$T_{1/2}$ (h)	6.27	6.79	6.25	8.33	6.43	6.88	6.68	6.91 ± 0.98	6.66 ± 0.22

T_{max}, peak time; C_{max}, maximal concentration; AUC_{last}, area under the time-concentration curve in serum between 0 and last observation; $AUMC_{last}$, area under first-moment curve; MRT_{last}, mean residence time, $T_{1/2}$, terminal half-life.

Table S3. Parameter estimates for unconjugated BPS from non-compartmental analysis by individual and sex.

Parameter	A	B	C	D	E	F	G	Male (n = 4)	Female (n = 3)
Dose (nmol)	2498	3101	2667	2550	2732	1827	1906	2704 ± 274	2155 ± 501
T_{max} (h)	0.50	1.00	0.50	0.50	1.00	0.25	0.25	0.63 ± 0.25	0.50 ± 0.43
C_{max} (nM)	7.34	8.48	10.9	10.1	9.14	10.4	14.0	9.21 ± 1.61	11.2 ± 2.55
AUC_{last} (nM × h)	35.3	41.7	38.1	42.5	40.7	42.3	38.0	39.4 ± 3.34	40.3 ± 2.18
$AUMC_{last}$ (nM × h^2)	212	272	206	262	245.7	250	185	238 ± 33.7	227 ± 36.2
MRT_{last} (h)	6.02	6.53	5.41	6.15	6.04	5.91	4.88	6.03 ± 0.47	5.61 ± 0.64
$T_{1/2}$ (h)	4.18	4.54	3.76	4.27	4.20	4.10	3.39	4.19 ± 0.32	3.90 ± 0.44

T_{max}, peak time; C_{max}, maximal concentration; AUC_{last}, area under the time-concentration curve in serum between 0 and last observation; $AUMC_{last}$, area under first-moment curve; MRT_{last}, mean residence time, $T_{1/2}$, terminal half-life.

Table S4. Comparison of pharmacokinetic parameters from non-compartmental analysis between unconjugated BPS (n = 7) and unconjugated BPA (n = 14).

Parameter	Unconjugated BPS (This study)	Unconjugated BPA (Thayer et al., 2015)
Dose (nmol)	2469 ± 455	35120 ± 6791
T_{max} (h)	0.57 ± 0.31	1.33 ± 0.51
C_{max} (nM)	10.1 ± 2.14	6.47 ± 3.19
AUC_{last} (nM × h)	39.8 ± 2.72	23.5 ± 6.04
$AUMC_{last}$ (nM × h^2)	233 ± 32.3	106 ± 32.8
MRT_{last} (h)	5.85 ± 0.54	4.67 ± 0.90
$T_{1/2}$ (h)	4.06 ± 0.38	3.24 ± 0.63

Each value is presented as mean ± standard deviation of participants. T_{max}, peak time; C_{max}, maximal concentration; AUC_{last}, area under the time-concentration curve in serum between 0 and last observation; $AUMC_{last}$, area under first-moment curve; MRT_{last}, mean residence time, $T_{1/2}$, terminal half-life.

국문초록

경구 투여에 따른 비스페놀 S 의 인체 내 약물동태학 연구

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비스페놀 S (BPS)는 비스페놀 (BPA)의 대체물질로서 폴리카보네이트 플라스틱, 에폭시 수지, 감열지 제조에 널리 사용되고 있다. BPS의 유해건강영향과 광범위한 노출이 보고됨에도 불구하고, 주요 노출경로인 경구 노출에 따른 동물 또는 인체 내 거동에 대한 정보는 알려진 바 없다. 본 연구의 목적은 BPS의 단회 경구 투여 후 인체 내에서의 흡수, 분포, 대사 및 배설에 대한 특성을 파악하고, 약물동태학적 모델을 구축하여 이를 설명하는 것이다. 이를 위해 7 명의 건강한 성인 피실험자에게 체중 당 8.75 μg의 중수소가 치환된

BPS (d_4-BPS)를 경구 노출시킨 후 48 시간 동안 지정된 시점에서 혈청 및 소변 시료를 수집하였으며, HPLC-MS/MS를 이용하여 d_4-BPS 원물질 및 대사체의 농도를 측정하였다. 시간에 따른 혈중 농도 및 누적소변배출량 프로파일을 바탕으로 약물동태학 분석을 수행하였으며, 약물동태학적 모델을 구축하였다. 비구획분석 (non-compartmental analysis) 결과 total d_4-BPS는 투여 후 1 시간 이내에 빠르게 위장관을 통해 흡수되며, 약 6.8 시간의 반감기를 지니면서 소변으로 배출되었다. 48 시간동안 남성의 경우 투여 용량 대비 평균 92%의 d_4-BPS가 소변으로 배출되었으며, 여성의 경우 평균 70%가 배출되었다. 또한 BPS의 인체 내 거동을 설명하기 위해 2-컴파트먼트 모델을 구축하였는데, 이 모델은 시간에 따른 혈중 농도 및 누적 소변배출량 프로파일을 잘 예측하였으며, 이 모델을 이용하여 산출한 약물동태학적 파라미터는 비구획분석을 통해 추정한 파라미터와 유사한 값을 나타내었다. 본 연구의 결과를 기존의 BPA에 대한 인체 약물동태학 연구결과와 비교하였을 때 BPS는 BPA보다 인체에 약간 더 긴 시간 동안 체류한다고 볼 수 있다. 본 연구는 인체 내에서 BPS의 흡수, 분포, 대사 및 배설에 관한 정보를 제공하며, 구축된 약물동태학적 모델은 바이오모니터링 자료를

이용한 BPS의 경구 노출 용량을 역산하는데 활용함으로써 보다 정확한 노출 평가에 기여할 수 있을 것이다.

주요어: 비스페놀 S, 경구 투여, 인체 약물동태학, 흡수 · 분포 · 대사 · 배설, 약물동태학 모델

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