보건학석사 학위논문

Human pharmacokinetic profiles of propyl paraben after oral administration

경구 투여에 따른 프로필 파라벤의 인체 내 약물동태학적 프로파일 연구

2017년 2월

서울대학교 보건대학원
환경보건학과 환경보건학 전공
신 초 롱

Abstract

Human pharmacokinetic profiles of propyl paraben after oral administration

Chorong Shin

Department of Environmental Health
Graduate School of Public Health
Seoul National University

Parabens are commonly used as antimicrobial preservatives in consumer products. Because of possible endocrine disrupting activities, their safety has become a public concern. While pharmacokinetic studies on parabens have been reported in animal, it is not well known to the fate of parabens in human body following oral exposure. Thus, the aim of this study was to determine pharmacokinetic characteristics of propyl paraben (PP) in male subjects following single oral administration of 0.6 mg/kg bw of deuterium labeled-PP. Blood and urine were collected for 72 h, and concentration of free PP and metabolites; conjugates and hydrolysates were measured. In blood, free PP had 2 distinct phases in time-profile, while the metabolites followed first-order kinetics. The fraction of urinary excretion for total PP (free plus conjugates)

was 8.6 %, whereas 38.7% of the administered dose was recovered in urine as sum of overall substances. The predominant metabolite excreted was *p*-hydroxyhippuric acid. Based on time-profiles, we constructed a multi-compartmental model and estimated kinetic parameters. PP was rapidly absorbed after oral ingestion and systemically distributed in humans. However, most of PP was likely to be metabolized prior to excretion, and rarely present in free form. The results of this study may be used for characterize PP exposure in the Korean population based upon biomonitoring data.

Keywords: paraben, oral exposure, pharmacokinetics, human metabolism, urinary excretion fraction

Student Number: 2015-24054

Contents

List of Tables

List of Figures

Ⅰ. Introduction

Parabens are commonly used as antimicrobial preservatives in various consumer products and the primary exposure route in humans is the consumption of foodstuffs and pharmaceuticals, or dermal application of cosmetics (Ferguson et al. 2016; Guo and Kannan 2013; Liao et al. 2013; Moreta et al. 2015). Because of the widespread use, parabens are ubiquitously found in the environment (Evans et al. 2016; Kasprzyk-Hordern et al. 2008; Peng et al. ; Rudel et al. 2003; Tran et al. 2016; Wang and Kannan 2016; Zhang et al. 2015) and have been detected even in human breast milk, placenta and cord blood (Pycke et al. 2015; Towers et al. 2015; Ye et al. 2008). Thus, general population are inevitably exposed to parabens in their daily life. Methyl- (MP), ethyl- (EP), *n*-propyl (*n*-PP) and *n*-butyl paraben (*n*-BP) are the most frequently detected compound. The detection rates for MP, EP, *n*-PP, and *n*-BP in urine among the Korean population were 97.7%, 97.2%, 96.7%, and 83.5%, respectively (Kang et al. 2016).

Although parabens have been considered safe, several studies have raised endocrine disruption of these compounds (Gomez et al. 2005; Pop et al. 2016; Prusakiewicz et al. 2007) and the estrogenic potency seems to elevate with the length of the alkyl chain (Gao et al. 2016). Adverse effects on sperm production are reported in male rats following oral administration of *n*-BP and *n*-PP (Oishi,

2001; Oishi, 2002). In humans, there is a weak but significant relationship between urinary paraben concentrations and sperm DNA damage (Meeker et al. 2011). Furthermore, it is suggested that lower doses of n-BP may increase the risk of breast cancer by stimulating breast cancer cell proliferation (Pan et al. 2016), and urinary MP or EP levels are associated with several oxidative stress related biomarkers (Kang et al. 2011). The Ministry of Food and Drug Safety of Korea (MFDS) permits the use of parabens at levels up to 0.4% for a single paraben and 0.8% for mixtures of all parabens in cosmetic ingredients while the use of *iso*-PP, *iso*-BP, BzP and PeP is banned (MFDS, 2015). The European Union Scientific Committee on Consumer concluded that the safety of PP and BP is uncertain due to lack of data and thus, the maximum allowed concentration is adjusted to 0.14% for PP or BP in cosmetic products (European Parliament, 2014a; European Parliament, 2014b). In foods, the acceptable daily intake (ADI) of parabens for the sum of MP and EP is 10mg/kg bw/day (JECFA, 2007).

Parabens are easily absorbed following oral or dermal exposure, hydrolyzed by *p*-hydroxybenzoic acid (pHBA) and *p*-hydroxyhippuric acid (pHHA), and excreted in urine as free form and glucuronide, as well sulfate conjugates (Abbas et al. 2010; Gopalakrishnan et al. 2016; Janjua et al. 2007; Soni et al. 2005). The metabolic pathways of parabens in humans until elimination via urine are shown in Fig. 1. Efficiency of hydrolysis of parabens differ depending on the alkyl chain length (Ozaki et al. 2013).

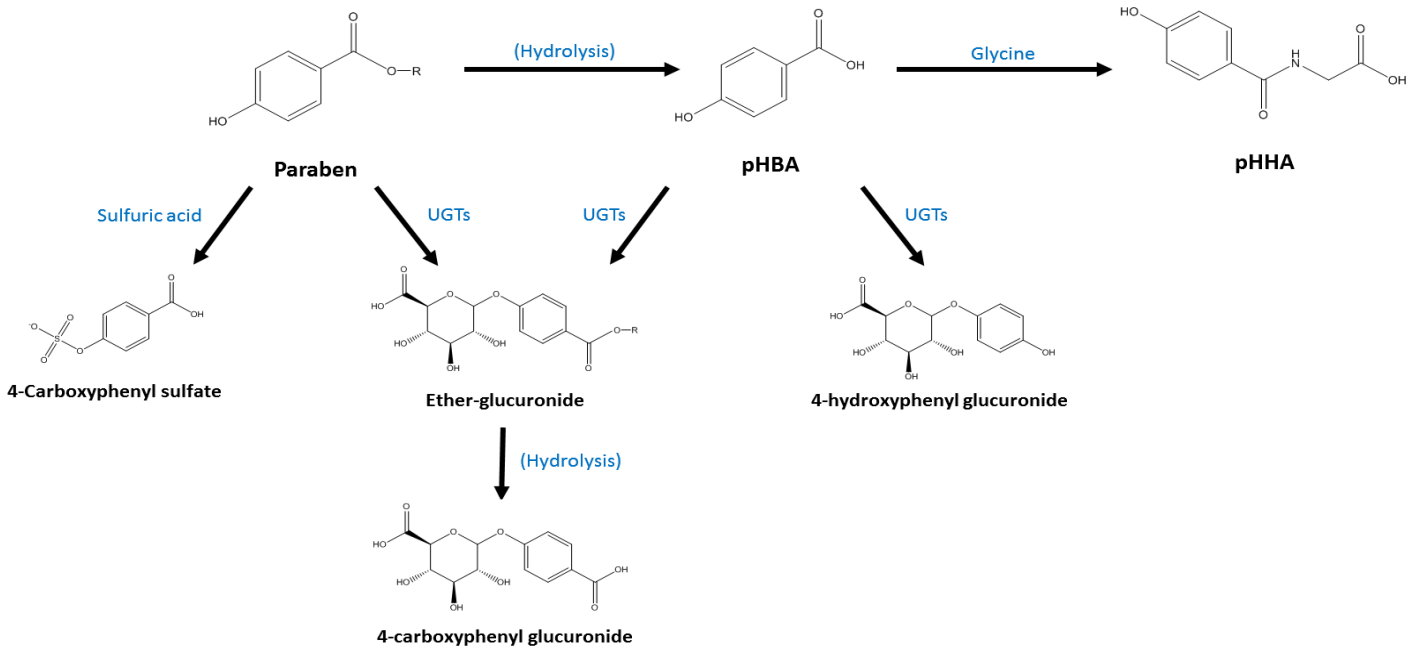

Fig. 1. Metabolic pathways of parabens in human. Adapted from Abbas et al. (2010)

Abbreviation: pHBA – para-hydroxybenzoic acid; pHHA – para-hydroxyhippuric acid; UGT – UDP-glucuronosyltransferases.

Several data on the pharmacokinetics of parabens have been reported in animal (Aubert et al. 2012; Gazin et al. 2013; Kim 2014; Kiwada et al. 1979; Kiwada et al. 1980; Kiwada et al. 1981; Mathews et al. 2013), whereas research on human is quite limited. After topical application of BP in male volunteers, a rapid skin penetration and systemic uptake were confirmed (Janjua et al. 2007). The urinary excretion factors (F_{ue}) for MP and BP following oral administration were reported by Moos et al. (2015). However, regarding to F_{ue}, it should be assumed that a simple steady-state kinetics for parabens. Moreover, although PP is the most commonly detected substance in personal care products with MP and potential to be more toxic because of the longer chain length, any kinetic information for PP had been not suggested based on human data. Therefore, the elaborate study is required on the kinetics for PP in humans.

The aim of this study was to determine pharmacokinetic characteristics of PP and its metabolites in male subjects after a single oral dosage. Thus PP and the metabolites were measured in serum and urine, and the compartmental pharmacokinetic model was developed to describe the absorption, distribution, metabolism and elimination of PP.

Ⅱ. Materials and methods

1. Experimental design

Five healthy volunteers were orally applied to the deuterated *n*-propyl paraben (PP-d4). All participants (n=5) were male and lived in Seoul, Korea. 400 mg of PP-d4 was dissolved in 1 mL ethanol and 100 μL of solution was spiked into a snack, which was fed to subjects. The administered dose was below the acceptable daily intake (ADI) of 10 mg/kg bw/day for the sum of MP and EP, instead of PP, because PP do not include the group ADI (JECFA, 2007). Blood and urine samples were monitored over 72 h and the first samples (T_0) before exposure were collected. The volume of the each urine samples was measured and all samples were stored at -70 °C until analysis. The present experiment was approved by the Seoul National University Institutional Review Board (SNUIRB # 1508/001-007), and all participants signed informed consent forms before participating.

2. Chemicals

n-propyl 4-hydroxybenzoate-2,3,5,6-d_4 (PP-d4, 98.8%) and *p*-hydroxybenzoic-2,3,5,6-d_4 (pHBA-d4, 99%) were purchased from CDN Isotopes Inc. (Point-Claire, Quebec). *p*-hydroxyhippuric acid (HHA, >99%) was obtained from Bachem (Torrance, CA). $^{13}C_6$-methyl-4-hydroxybenzoate ($^{13}C_6$-MP) and $^{13}C_6$-propyl-4-hydroxybenzoate ($^{13}C_6$-PP) were used as internal standards and purchased from Sigma-Aldrich (St. Louis, MO). Acetonitrile, methanol, and water (HPLC grade) were purchased from J. T. Baker (Center Valley, PA) and acetic acid (HPLC grade) was obtained from Fisher Chemical Co. (Fair Lawn, NJ). Formic acid (98+%) were purchased from Acros organics Co. (Morris Plains, NJ) and ammonium acetate ($\geq$ 97%), β-Glucuronidase and sulfatase from *Helix pomatia* were purchased from Sigma-Aldrich (St. Louis, MO).

3. Analytical procedure

3.1 Sample preparation

The biological samples were prepared by the enzyme hydrolysis method as previously described (Lee et al. 2013) with a minor modification. In brief, 500 μL of an aliquot of serum or urine was mixed with 10 μL of the internal standard solution (a mixture of $^{13}C_6$-MP and $^{13}C_6$-PP), and 100 μL of 1 M ammonium acetate was added. The enzymatic treatments were performed by adding 20 μL of β-glucuronidase/sulfatase and samples were incubated for 2 h at 37 °C. The samples were acidified using 2 mL of 0.1 M formic acid and loaded on the solid phase extraction (SPE) cartridge, Oasis HLB (polymeric reversed phase, 30 mg/3cc; Waters, Milford, MA), which had been conditioned with 3 mL of acetonitrile and 3 mL of deionized water. Absorbed paraben and metabolites were washed with 10% methanol and dried for 30 min at room temperature, and then eluted with 3 mL of methanol. The eluates were evaporated until dryness under a nitrogen flow and reconstituted in 100 uL of a 70:30 (*v*:*v*) water-methanol solution. Concentrations of unconjugated PP-d4 in serum and urine were determined following the same methodology described above without the enzymatic hydrolysis step.

3.2 Determination of PP and metabolites using HPLC-MS/MS

Measurement of PP and the metabolites in serum and urine was performed by HPLC-MS/MS with Nexera autosampler and pump (Shimadzu, Kyoto, Japan) coupled to API 4000 tandem mass spectrometer (AB Sciex, Framingham, MA) using a electrospray ionization source in negative mode (ESI－). The chromatographic separation was carried out on a Capcell pak ACR column (2.0 × 150 mm, 3 um) from Shiseido Co., LTD (Tokyo, Japan) using gradient mode. The mobile phase composition 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; 2.5-8.0 min, 45%-60% B; 8.0-9.0 min, 60%-90% min; 9.0-10.6 min, 90% B and return to 30% B in 10.6-13.0 min. The flow rate was 0.2 mL/min and 5 μL of each sample was injected into the UPLC system. The MS and electrospray ionization parameters were optimized separately for each analyte by direct infusion of standards using the Analyst 1.5.2 software (AB Sciex) (Table S1).

Quantification was based on peak areas relative to the stable isotope-labeled internal standards. Because the dosing substance in this study was deuterium-labeled PP (PP-d4), we used $^{13}C_6$-labeled-PP as an internal standard, while $^{13}C_6$-labeled-MP was used for the quantification of pHBA-d4 and pHHA-d4. Method validation included analysis of target chemicals into serum and urine samples on 3 different days at 3 levels of concentration. The intra- and inter-day

accuracy and precision were ranging from 87 to 114% and 3 to 12% RSD, respectively (Table S2). The limit of quantitation (LOQ) was defined as a signal-to-noise ratio of 9 and estimated from 0.2 ng/mL (for PP-d4 and pHBA-d4) to 5 ng/mL (for pHHA-d4) in both of biological samples.

For quality control, analysis of blank, standard-spiked samples (spiked-QC) and pooled human serum or urine samples from this study (pooled-QC) was carried out during every sample set. In blank, any labeled analytes were not observed as a quantifiable level. For all analytes and both of the biological samples, the recovery in the spiked-QC samples was between 84% and 109% and the coefficient of variation for pooled-QC samples varied from 8% to 10% (data not shown).

4. Pharmacokinetic analysis

4.1 Estimation of the kinetic parameters

Based on measured data, we calculated the descriptive kinetic parameters (*i.e.*, area under the curve [AUC], C_{max} and T_{max}). Maximum concentration (C_{max}) and peak time (T_{max}) were obtained directly by the time profile in serum. The elimination rate constant (K) was evaluated by log-linear regression of the terminal phase of time profile for each substance, and the elimination half-life ($t_{1/2}$) was calculated as ln2/K. $AUC_{0-\infty}$ and $AUMC_{0-\infty}$ was determined according to the trapezoidal method, and mean residence time (MRT) was estimated as $AUMC_{0-\infty}/AUC_{0-\infty}$ under the non-compartmental analysis. Total clearance (CL_{total}) and the volume of distribution (V_d) were calculated from $F \cdot$Dose/AUC and CL_{total}/K, respectively.

4.2 Development of the pharmacokinetic model

As shown in Fig. 1, metabolism of PP was classified into 2 types; Met 1 (formation of conjugated metabolite of PP with glucuronidise and sulfuric acid) and Met 2 (formation of the hydrolysates, pHBA and pHHA), and these metabolic pathways were considered in the structure of model. Therefore, a multi-compartment model was constructed to investigate the pharmacokinetics of PP after oral administration (Fig. 2). It was assumed that the rate of absorption from gastrointestinal (GI) tract and elimination via urine might be explained as first-order kinetics. Based on observed data in serum, the formation and disposition of 2 types of metabolites were supposed to follow first-order kinetics, too. For free PP (not metabolized in the body), two-compartment model could be more suitable since there were 2 distinct phases at time profile of free PP.

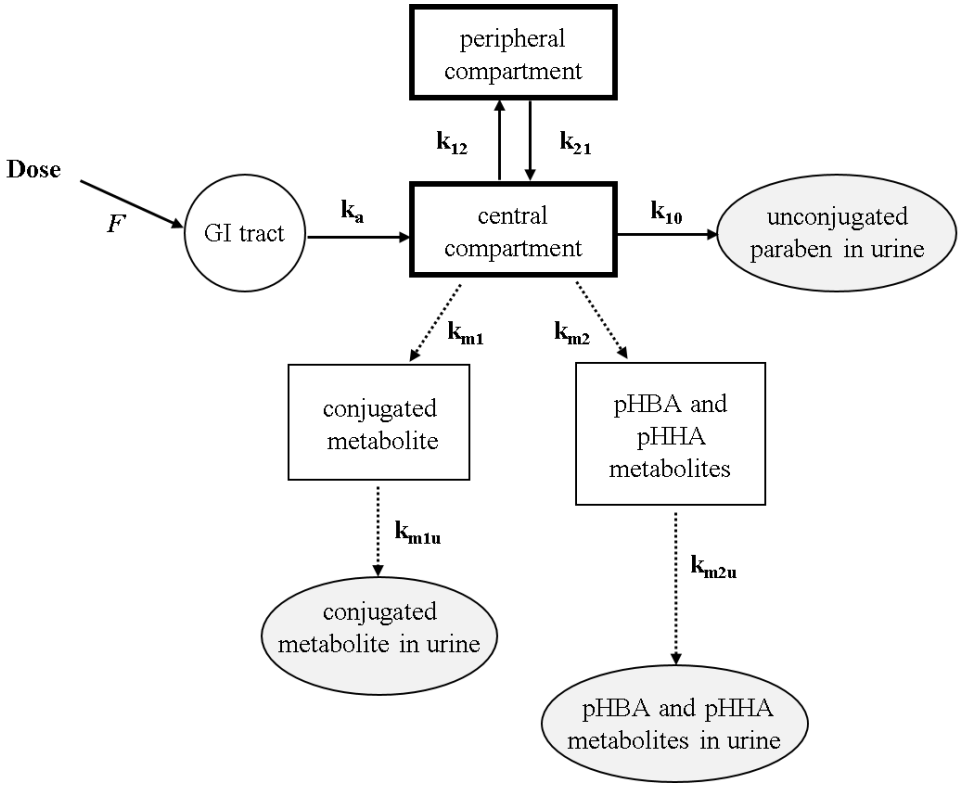

Fig. 2. Structure of the pharmacokinetic model for PP and its metabolites after oral administration

Abbreviations: *F* – fraction of dose absorbed (bioavailability); GI tract – gastrointestinal tract; k_a – PP absorption; k_{12} – PP distribution from central compartment (blood) to peripheral compartment; k_{21} – reabsorption from peripheral compartment to blood; k_{10} – PP elimination at blood; k_{m1} – PP → conjugated metabolite formation; k_{m1u} – conjugated metabolite elimination; k_{m2} – PP → pHBA and pHHA metabolites formation; k_{m2u} – pHBA and pHHA metabolites elimination

4.3 Model optimization

We fitted the multi-compartment model to measured serum and urine data using Berkeley Madonna (version 8.3.9, University of California at Berkeley). Ordinary differential equations were used to describe the rate of change for each component in model and solve using the fixed step size integration algorithm, Fourth-order Runge-Kutta algorithm (RK4), available in Berkeley Madonna. Parameter estimation was performed using all measured data for the unconjugated PP and metabolites. The initial values in optimization procedures were obtained from the preliminary analyses for the unconjugated PP such as non-compartment analysis, or one-compartmental models according to the residual method.

4.4 Model validation

The final PK model was tested to validate the performance with the separated data sets against those used for parameterization. Data sets used here for validation of the PK model were obtained from another controlled oral-dosing study for PP (MFDS, 2013. Report #. 13162MFDS776) that 7 male subjects were exposed to PP in a similar manner which was described in the present study. The administered dose was 2.5 mg/kg bw, which is 5-fold higher than that of the present study, and blood and urine samples were collected by 8 and 24 h after administration. Validation of the PK model was performed as follows. First, kinetic parameters optimized with our observations were fixed. Only amount of the administered dose was adjusted from 0.6 to 2.5 mg/kg bw. After that, model prediction was compared with measured data from MFDS (2013) to assess the fitting status.

Ⅲ. Results

1. Subject characteristics

The demographic characteristics for 5 volunteers were presented in Table 1. The average age, body weight, height and body mass index (BMI) of the subjects were 27 years (range 23－33), 69 kg (range 62－74), 175 cm (range 170－180) and 22.4 (range 21.5－23.7), respectively.

Table 1. Demographic characteristics of the study participants.

Subject ID	Age (y)	Height (cm)	Body weight (kg)	BMI
A	33	179	72	22.5
B	26	180	71	21.9
C	25	171	66	22.6
D	29	170	62	21.5
E	23	177	74	23.7
Mean ± SD	27 ± 3	176 ± 4	69 ± 5	22.4 ± 0.8

All participants (n=5) were male.

2. Serum pharmacokinetic profiles of PP and its metabolites

The serum concentration-time profiles for PP and its metabolites after oral administration were shown in Fig. 3. It was confirmed that any target compound was not detected in samples collected before administration. Unconjugated PP was present in serum with the lowest level compared with other metabolites. Met1 (conjugated PP) was detectable at 24 h after administration, while pHBA was detected until 12 h. For pHHA and the unconjugated PP, they were detected up to 4 h after administration but the detectable range slightly varied between the subjects. Neither parent compound nor the metabolites was detected in any subject since 48 h. Therefore, PP was completely eliminated from blood within 48 h after oral administration in our subjects. It was worthy to note that the 2 distinct phases were observed for the unconjugated PP (black square); the early phase with a rapid decline until 1 h, and the second phase with a gradual and slow decline until 6 h. Thus a two-compartment model was appropriate to predict time profile for the free PP. On the other hand, the metabolites appeared to follow first-order kinetics for formation and disposition, and one-compartment model was enough for Met1 and Met2. Following these observations, the structure of the model was confirmed as a multi-compartment model (Fig. 2).

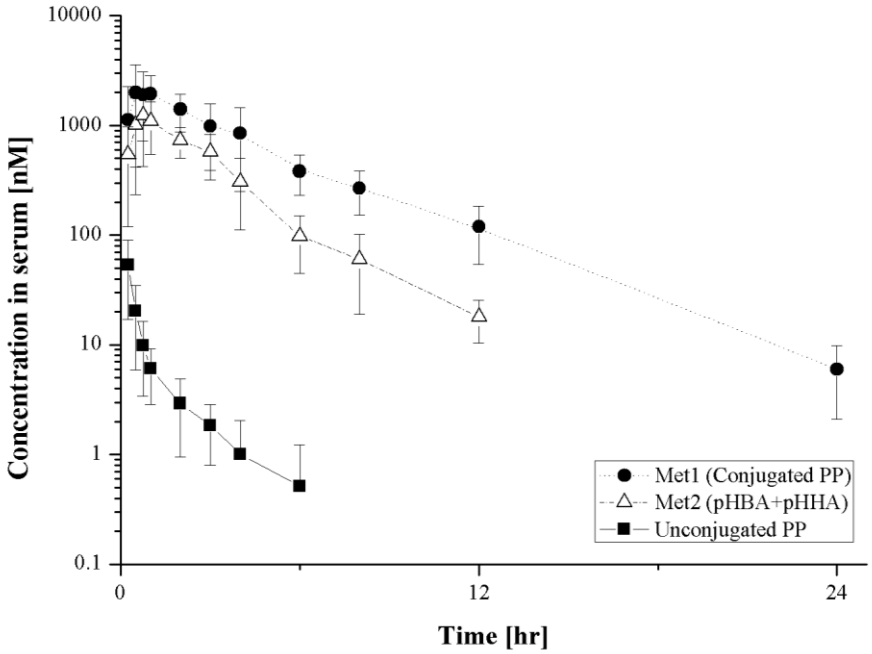

Fig. 3. Time profiles of PP and its metabolites in serum after oral administration of PP. Met1; conjugated metabolite of PP, Met2; sum of the hydrolysates, pHBA and pHHA. Each symbol and error bar represents the arithmetic mean and the standard deviation of each substance at a given time point (n=5)

3. Non-compartmental analysis

Using time profiles in serum, estimation of the kinetic parameters under the non-compartmental analysis was performed. As shown in Table 2, the unconjugated PP was present in serum at a low level (C_{max}, 54 ± 37 nM) than other metabolites, and the highest C_{max} was observed in Met1 (C_{max}, 2766 ± 896 nM). All substances reached their maximum concentration within 2 h. Half-life ($T_{1/2}$) for each compound was calculated. In blood, Met1 was eliminated with a half-life of 2.9 h, while pHBA (2.4 h), pHHA (1.3 h) and unconjugated PP (0.9 h) were eliminated slightly faster. Area under the curve (AUC) of Met1 was about 2.5-fold higher than that of Met2, and mean residence time (MRT) was highest in Met1, as well.

Table 2. Parameter estimates from non-compartmental analysis

	C_{max} (nM)	T_{max} (h)	$T_{1/2}$ (h)	$AUC_{0-\infty}$ (nM·h)	$AUMC_{0-\infty}$ (nM·h^2)	MRT (h)
Unconjugated PP	54 ± 37	0.25 ± 0	0.9	33	43	1.3
Met1	2766 ± 896	1.4 ± 1.1	2.9	8725	36826	4.2
Met2	1485 ± 518	1.4 ± 1.1	1.8	3561	9155	2.6
pHBA	755 ± 436	1.7 ± 1.4	2.4	2322	7919	3.4
pHHA	881 ± 488	1.2 ± 1.1	1.3	1416	2740	1.9

Met1, conjugated PP; Met2, sum of pHBA and pHHA; C_{max}, peak concentration; T_{max}, peak time; $T_{1/2}$, terminal half-life; $AUC_{0-\infty}$, area under the serum concentration-time curve between 0 and ∞; $AUMC_{0-\infty}$, area under first-moment curve between 0 and ∞; MRT, mean residence time; C_{max} and T_{max} values were expressed as arithmetic mean ± standard deviation (n=5).

4. Urinary elimination kinetics of PP and its metabolites

Profiles of the cumulated excretion amount of PP and its metabolites were displayed in Fig. 4. PP was mainly excreted as a metabolized form rather than unconjugated form. Within metabolites, the level of excreted amount into urine was higher in Met2 than Met1. After 72 h of administration, there was no detection for any compound in urine, thus the excretion of PP, including metabolites, could be regarded as being complete by 72 h in our subjects.

Overall, an average of 39% of administered dose was recovered in urine by 72 h after administration (Table 3)**.** pHHA appeared to be a predominant metabolite among all participants with a share of 23.2%, and 7.0% of the dose were excreted as the other hydrolysate, pHBA. The conjugated PP (Met1) was recovered 8.5% of the applied dose, whereas only 0.1% of the dose was excreted as a free species without metabolism. Thus, the fraction of urinary excretion (F_{ue}) for total PP (free plus conjugates) was approximately 8.6%.

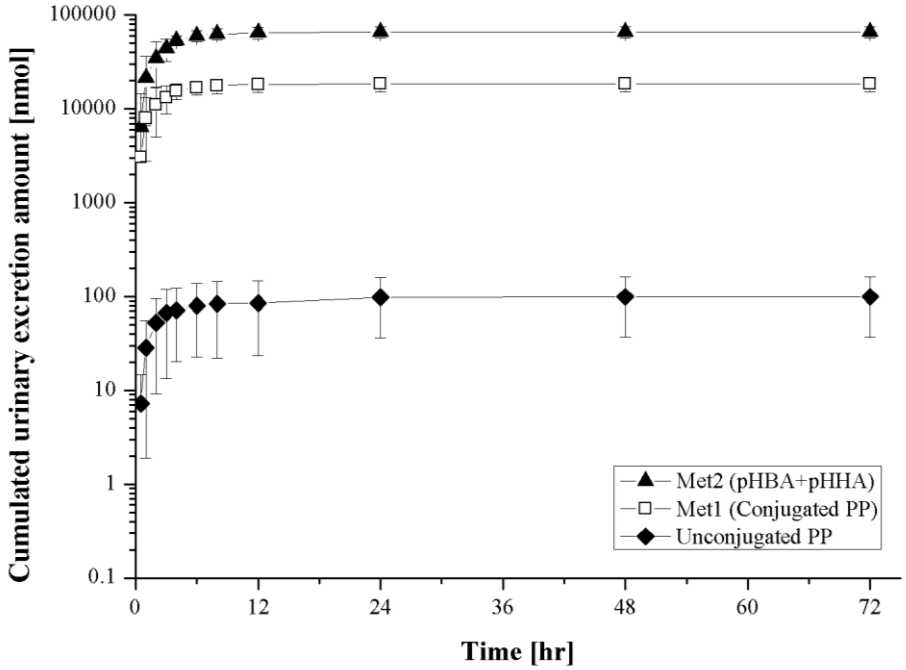

Fig. 4. Cumulative urinary excretion of PP and its metabolites after oral administration of PP. Each symbol and error bar represents the arithmetic mean and the standard deviation of each substance at a given time point (n=5)

Table 3. The fraction of urinary excretion (F_{ue}, %) of PP and its metabolites in the study participants after oral administration.

	Subject ID					Mean ± SD
	A	B	C	D	E	
Unconjugated PP	0.09	0.02	0.01	0.05	0.05	0.05 ± 0.03
Met1	9.4	6.8	9.3	9.9	7.2	8.5 ± 1.4
Met2						
pHBA	7.3	6.4	8.3	6.8	6.0	7.0 ± 0.9
pHHA	23.2	24.4	27.5	23.1	17.8	23.2 ± 3.5
Overall Σ	39.9	37.6	45.2	39.9	31.1	38.7 ± 5.1

Values were calculated as percent of administered dose on a molar basis. Met1, conjugated PP; Met2, sum of pHBA and pHHA; SD, standard deviation.

5. Pharmacokinetic modeling

Model optimization was performed under the multi-compartmental structure, which was illustrated in Fig. 2. The initial values for the rate constants (*i.e.*, k_a, k_{10}, k_{12}, and k_{21}) were obtained from a preliminary analysis. We fixed these values and fit the PK model to time profile data using Berkeley Madonna. As shown in Fig. 5, the model represented good prediction for time profiles of PP and its metabolites. Using this model, we determined the kinetic parameters for PP after oral administration (Table 4). Based on the estimates, we concluded that PP was rapidly absorbed (k_a = 6) and distributed (k_{12} = 2.5), while the rate of reabsorption from peripheral compartment was slower (k_{21} = 0.55). After absorption, the parent compound was quickly metabolized and Met2 (pHBA and pHHA) were formed faster (k_{m2} = 25) than Met1 (conjugated PP) (k_{m1} = 6). Besides, the elimination rate of Met2 was slightly faster (k_{m2u}=0.45) than that of Met1 (k_{m1u} = 0.3). However, the unmetabolized PP was eliminated quite slowly (k_{10} = 0.02) than other metabolites.

The final model was validated with observed data from MFDS (2013) with a single oral administration of 2.5 mg/kg bw of PP-d4 to male volunteers (n=7). The kinetic parameters in Table 4 were fixed and only the administered dose was altered. After that, model prediction and time profiles were compared. As a result, our model provided good fits to the different time profiles, as well (Fig. 6).

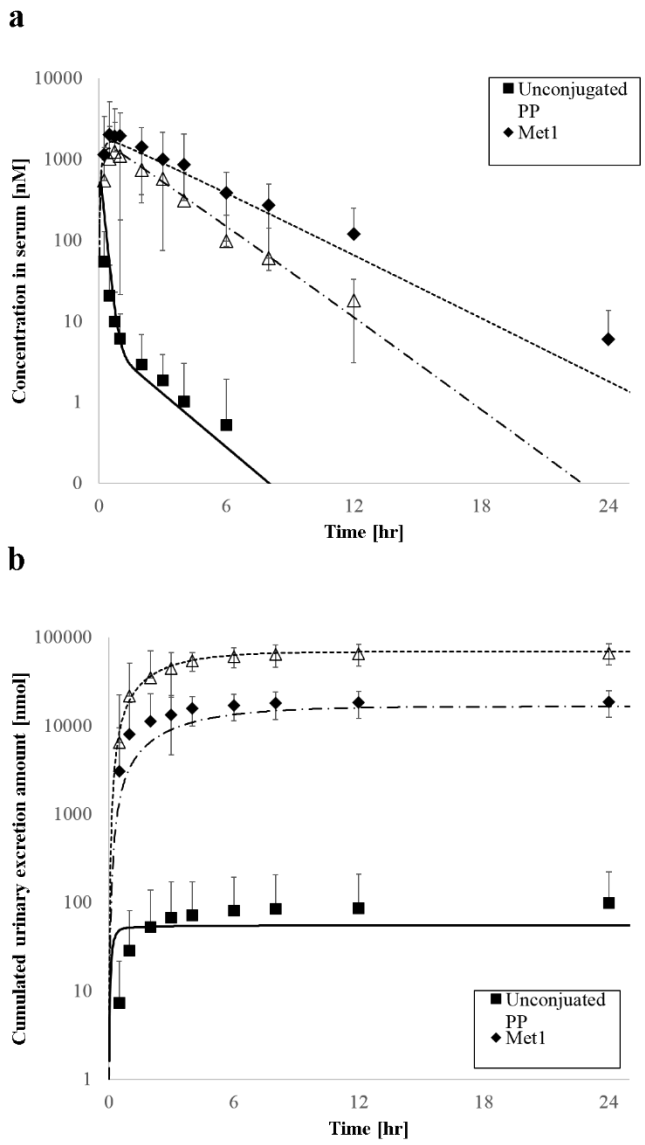

Fig. 5. Model optimization. (a) PK model prediction on serum concentration-time profile, (b) PK model prediction on cumulated urinary excretion amount-time profile. Model fitness status showed that PP and the metabolites were fitted well by the PK model developed in this study. Each symbol and error bar represents the arithmetic mean (AM) and 95% upper limits of the AM (1.96-fold of standard deviation) of each substance at a given time point (n=5)

Table 4. Kinetic parameter estimates of the PK model developed in this study.

Parameter	Notes	Estimates
F	Fraction of dose absorbed (bioavailability)	0.39
k_a	PP (GI tract) → PP (blood)	6
k_{12}	PP (blood) → PP (peripheral)	2.5
k_{21}	PP (peripheral) → PP (blood)	0.55
k_{10}	PP elimination from blood via urine	0.02
k_{m1}	PP → Met1 (conjugated PP) formation	6
k_{m2}	PP → Met2 (pHBA and pHHA) formation	25
k_{m1u}	Met1 elimination from blood via urine	0.3
k_{m2u}	Met2 elimination from blood via urine	0.45
V_{pb}	Volume of distribution for PP	20
V_{m1}	Volume of distribution for Met1	8
V_{m2}	Volume of distribution for Met2	38

Units of the reaction rate constant and volume of distribution are h^{-1} and L, respectively. Met1, conjugated PP; Met2, sum of pHBA and pHHA.

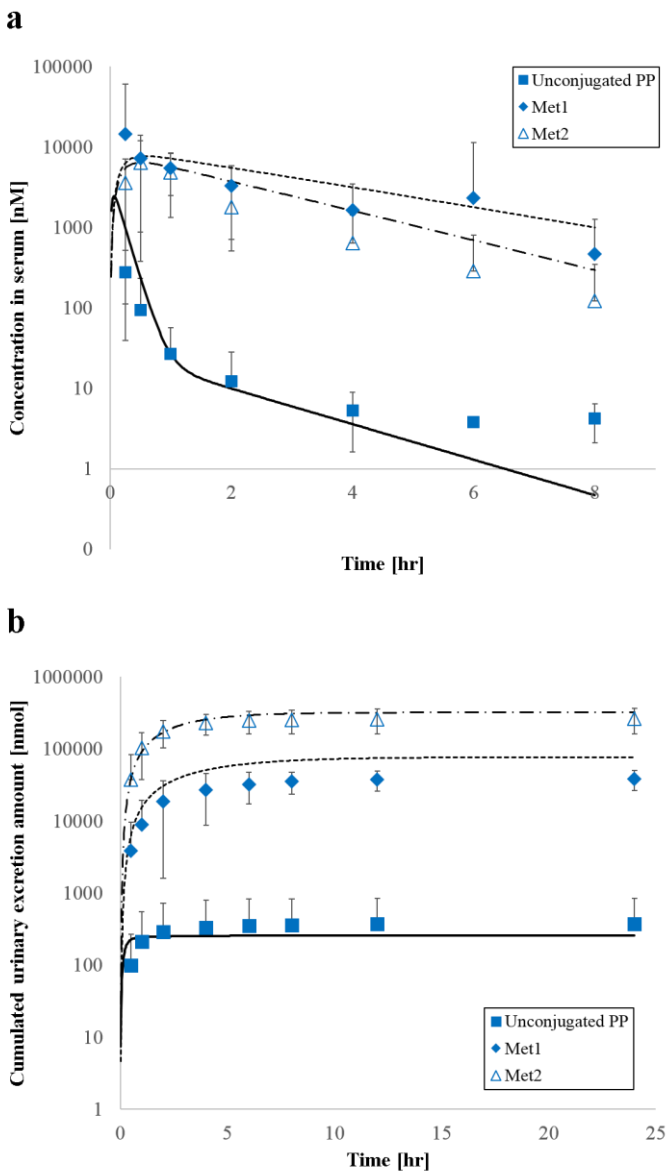

Fig. 6. Model validation. (a) PK model prediction on serum concentration-time profile, (b) PK model prediction on cumulated urinary excretion amount-time profile. The developed PK model in this study represents good fits to the measured PP and the metabolites from MFDS (2013). Each symbol and error bar represents the arithmetic mean (AM) and 95% upper limits of the AM (1.96-fold of standard deviation) of each substance at a given time point from MFDS (2013) (n=7)

Ⅳ. Discussion

In the present study, we investigated the time profiles of PP and its metabolites in serum and urine after oral administration from a controlled dosing study. Based on the observations, a human PK model, which is composed of a multi-compartment, was developed to describe absorption, distribution, metabolism and elimination of PP following oral exposure.

Our results show that PP was rapidly absorbed and systemically distributed in human after oral exposure, which is consistent with the findings from the other literatures for animal (Aubert et al. 2012; Gazin et al. 2013). With regard to level of serum concentration, the unconjugated PP were quite lower than the others. Thus most of PP is immediately metabolized into the conjugates or hydrolysates, and rarely present in blood as free form. Based on the parameter estimates from the PK model (Table 4), the forming and elimination rate for Met2 were considerably faster than Met1. Finally, PP was completely eliminated from blood within 48 h in our subjects.

The urinary elimination kinetics of PP and the metabolites were investigated (Fig. 4). For 72 h after administration, PP was totally excreted into urine. The overall fraction of total excreted biomarkers in urine was 39% and most amounts were metabolized forms rather than free form (Table 3). For total PP, which is commonly used as a urinary biomarker of PP, F_{ue} was 8.6%. We have

a similar observation in the previous studies in that the F_{ue} of parent paraben generally decreases with increasing length of the alkyl chain (Table 5). Additionally, the predominant metabolite was pHHA in the present study and this is consistent with results of the previous studies (MFDS, 2013; Moos et al. 2015). However, it seems that there is certain difference in metabolism and elimination of paraben between Korean and German population considering the F_{ue} of the metabolites. The F_{ue} of pHHA in German subjects after oral administration of MP and BP were 63.8 and 61.8%, respectively (Moos et al. 2015). In our study, only 21.4% of administered dose was recovered in urine as pHHA, which is 3-fold lower than that of the previous study. As a results, the overall F_{ue} including metabolites in the present study was approximately 2-fold lower than that of German. Thus, there is a probability to underestimate the intake dose of paraben for Korean population in the case of performing a dose reconstruction with urinary concentration of paraben using F_{ue} reported from the previous study.

Table 5. The fraction of urinary excretion (F_{ue}, %) of total paraben reported in the present study and previous studies.

Country	n	Gender	F_{ue} for total paraben[a]				Reference
			MP	EP	PP	BP	
Korea	7	Male	7.7 (5.6-10.5)	6.0 (3.0-8.4)	3.8 (2.9-5.5)		MFDS (2013)
	5	Male			8.6 (6.8-10.0)		This study
Germany	3	Male and female	17.4 (15.5-19.2)			5.6 (5.2-6.4)	Moos et al. (2015)

MP, methyl paraben; EP, ethyl paraben; PP, *n*-propyl paraben; BP, *n*-butyl paraben

[a] calculated as percent of administered dose on a molar basis and expressed as mean (range).

Several population studies reported the ratio of free species to total paraben (free plus conjugates) in urinary excretion amount and suggested that the decreasing share of free paraben with increasing chain length (Guidry et al. 2015; Ye et al. 2006). According to Ye et al. (2006), only 2% of PP was excreted in free form and Guidry et al. (2015) reported that free concentration comprised 0 – 5% of the total concentration in half of the samples analyzed for PP. However, the latter author implied evidences of contamination for levels of free PP and it could be possible because they measured unlabeled PP. In contrast, we quantified the deuterium labeled-PP within a set of controlled dosing without the possibility of contamination. As a result, only 0.5% of PP was excreted via urine as a free form in comparison with total PP. Because there are no additional studies about the ratio of free to total PP in human following controlled dosing experiment, we compared our findings with those for other paraben. Moos et al. (2015) reported that after oral administration in human subjects, 7.1 and 1.0% of total paraben were eliminated as a free species for MP and BP, respectively. The ratio of free BP to BP-glucuronide was measured following dermal application and only about 2% of BP was excreted as an unconjugated form (Janjua et al. 2008). Consequently, our observations concur with findings from the other studies in that the ratio of free to total paraben might be decreasing following increase of alkyl-chain length. Moreover, we could provide information for the disposition of unmetabolized PP in serum resulting in urinary excretion amounts using the PK model.

To comprehend biomonitoring data for parabens within the framework of risk assessment, understanding on the fate of parabens in human is certainly needed. In other words, a quantitative knowledge on human pharmacokinetics of paraben is essential to investigate paraben exposure using biomonitoring data. It might be an alternative approach to extrapolate results from animal to human, however, there are species-dependent differences between rodent and human including kinetic characteristics, and the dosage which applied to animal. Several studies have reported the inter-species differences in metabolism of paraben. Sulfated conjugates were predominates in rats (Gazin et al. 2013), whereas, almost the same amounts of sulfate and glucuronide conjugates were appeared in spot urine samples in human (Ye et al. 2006). Though more complicated physiologically based pharmacokinetic (PBPK) models for parabens with quantitative *in vitro* to *in vivo* extrapolation (IVIVE) were developed (Campbell et al. 2015), however, the model parameterization was primarily rely on extrapolation from experimental animal studies and many assumptions were required to applicate results of *in vitro* experiments into the model. Because of inadequate data set in animal studies, attempts to incorporate in vitro measures for paraben conjugation were failed to predict the human data and certain results between in vivo and in vitro were contrasted. Therefore, the multi-compartment PK model in the present study can exhibit a valuable alternative for integrating biomonitoring data into exposure and risk assessment.

The limitation for application of the developed PK model was that we do not predict the kinetics after dermal application, which is another primary route of exposure to PP. Thus, further studies are needed. Additionally, the administered dose in the present study may differ with the level of exposure to PP among general population in Korea. However, several animal studies have reported that the pharmacokinetics of paraben following various dose levels does not remarkably altered (Gazin et al. 2013; Mathews et al. 2013). Therefore, the results in the present study could be used for characterize PP exposure in the Korean population based upon biomarker.

Ⅴ. Conclusions

The present study investigated time profiles of PP and its metabolites for male subjects in serum and urine following oral administration, and constructed a multi-compartmental model. After optimization of the PK model, we estimated kinetic parameters for unmetabolized PP and metabolites. The results of this study can suggest that PP is rapidly absorbed and systemically distributed in humans after oral ingestion. Furthermore, most of PP is likely to be metabolized prior to excretion, and rarely present in free form in human body. With regard to metabolism, formation and elimination of hydrolysates, pHBA and pHHA, is faster than conjugates. The fraction of urinary excretion (F_{ue}) for total PP (free plus conjugates) and overall substances including metabolites was 8.6 and 38.7%, respectively. In conclusion, we can describe the absorption, distribution, metabolism and elimination of PP using the PK model.

Ⅵ. References

Abbas S, Greige-Gerges H, Karam N, Piet M-H, Netter P, Magdalou J. 2010. Metabolism of parabens (4-hydroxybenzoic acid esters) by hepatic esterases and UDP-glucuronosyltransferases in man. Drug Metabolism and Pharmacokinetics. 25 (6): 568-577.

Aubert N, Ameller T, Legrand J-J. 2012. Systemic exposure to parabens: Pharmacokinetics, tissue distribution, excretion balance and plasma metabolites of [^{14}C]-methyl-, propyl-and butylparaben in rats after oral, topical or subcutaneous administration. Food and Chemical Toxicology 50: 445-454.

Boberg J, Taxvig C, Christiansen S, Hass U. 2010. Possible endocrine disrupting effects of parabens and their metabolites. Reproductive Toxicology 30: 301-312.

Campbell JL, Yoon M, Clewell HJ. 2015. A case study on quantitative in vitro to in vivo extrapolation for environmental esters: Methyl-, propyl-and butylparaben. Toxicology 332: 67-76.

Evans WA, Davies PJ, McRae C. 2016. The occurrence of methyl, ethyl, propyl, and butyl parabens in the urban rivers and stormwaters of Sydney, Australia. Environmental Science: Water Research & Technology 2: 733-742.

Ferguson KK, Colacino JA, Lewis RC, Meeker JD. 2016. Personal care product use among adults in NHANES: Associations between urinary phthalate metabolites and phenols and use of mouthwash and sunscreen. Journal of Exposure Science and Environmental Epidemiology 1-7.

Gao Y, Ji Y, Li G, An T. 2016. Theoretical investigation on the kinetics and mechanisms of hydroxyl radical-induced transformation of parabens and its consequences for toxicity: Influence of alkyl-chain length. Water Research 91: 77-85.

Gazin V, Marsden E, Marguerite F. 2013. Oral propylparaben administration to juvenile male wistar rats did not induce toxicity in reproductive organs. Toxicological Sciences 136: 392-401.

Gomez E, Pillon A, Fenet H, Rosain D, Duchesne M, Nicolas J, et al. 2005. Estrogenic activity of cosmetic components in reporter cell lines: Parabens, UV screens, and musks. Journal of Toxicology and Environmental Health, Part A 68: 239-251.

Gopalakrishnan K, Teitelbaum SL, Lambertini L, Wetmur J, Manservisi F, Falcioni L, et al. 2017. Changes in mammary histology and transcriptome profiles by low-dose exposure to environmental phenols at critical windows of development. Environmental Research 152: 233-243.

Guidry VT, Longnecker MP, Aase H, Eggesbø M, Zeiner P, Reichborn-Kjennerud T, et al. 2015. Measurement of total and free urinary phenol and paraben concentrations over the course of pregnancy: Assessing reliability and contamination of specimens in the Norwegian mother and child cohort study. Environmental Health Perspectives 123: 705-711.

Guo Y, Kannan K. 2013. A survey of phthalates and parabens in personal care products from the United States and its implications for human exposure. Environmental Science & Technology 47: 14442-14449.

Janjua NR, Mortensen GK, Andersson A-M, Kongshoj B, Skakkebæk NE, Wulf HC. 2007. Systemic uptake of diethyl phthalate, dibutyl phthalate, and butyl paraben following whole-body topical application and reproductive and thyroid hormone levels in humans. Environmental Science & Technology 41: 5564-5570.

Janjua NR, Frederiksen H, Skakkebæk NE, Wulf HC, Andersson AM. 2008. Urinary excretion of phthalates and paraben after repeated whole-body topical application in humans. International Journal of Andrology 31: 118-130.

JECFA (Joint Food and Agricultural Organization/World Health Organization Expert Committee on Food Additives). 2007. Sixty-seventh report of the Joint FAO/ WHO expert committee on food additives. World Health Organization Technical Report Series 940.

Kang S, Kim S, Park J, Kim H-J, Lee J, Choi G, et al. 2013. Urinary paraben concentrations among pregnant women and their matching newborn infants of korea, and the association with oxidative stress biomarkers. Science of the Total Environment 461-462: 214-221.

Kang H-S, Kyung M-S, Ko A, Park J-H, Hwang M-S, Kwon J-E, et al. 2016. Urinary concentrations of parabens and their association with demographic factors: A population-based cross-sectional study. Environmental Research 146: 245-251.

Kasprzyk-Hordern B, Dinsdale RM, Guwy AJ. 2008. The occurrence of pharmaceuticals, personal care products, endocrine disruptors and illicit drugs in surface water in south wales, UK. Water Research 42: 3498-3518.

Kim P. 2014. Toxicokinetic modeling of ethyl paraben administered orally in rats. Korean Journal of Environmental Health Sciences 40: 407-412.

Kiwada H, Awazu S, Hanano M. 1979. The study on the biological fate of paraben at the dose of practical usage in rat. I. The metabolism and excretion of ethyl p-hydroxybenzoate (ethyl paraben) and p-hydroxybenzoic acid. Journal of Pharmacobio-Dynamics 2: 356-364.

Kiwada H, Awazu S, Hanano M. 1980. The study on the biological fate of paraben at the dose of practical usage in rat. Ⅱ. The pharmacokinetic

study on the blood concentration after the administration of ethyl paraben or p-hydroxybenzoic acid. Journal of Pharmacobio-Dynamics 3: 353-363.

Kiwada H, Awazu S, Hanano M. 1981. The study on the biological fate of paraben at the dose of practical usage in rat. Ⅲ. The effects of salicylic acid on the fate of ethyl paraben. Journal of Pharmacobio-Dynamics 4: 643-648.

Lee S-Y, Son E, Kang J-Y, Lee H-S, Shin M-K, Nam H-S, et al. 2013. Development of a quantitative analytical method for determining the concentration of human urinary paraben by LC-MS/MS. Bulletin of the Korean Chemical Society 34: 1131-1136.

Liao C, Liu F, Kannan K. 2013. Occurrence of and dietary exposure to parabens in foodstuffs from the United States. Environmental Science & Technology 47: 3918-3925.

Mathews JM, Brown SS, Patel PR, Black SR, Banks TT, Etheridge AS, et al. 2013. Metabolism and disposition of [^{14}C] n-butyl-p-hydroxybenzoate in male and female harlan Sprague Dawley rats following oral administration and dermal application. Xenobiotica 43: 169-181.

Meeker JD, Yang T, Ye X, Calafat AM, Hauser R. 2010. Urinary concentrations of parabens and serum hormone levels, semen quality parameters, and sperm DNA damage. Environmental Health Perspectives 119: 252-257.

MFDS (Ministry of Food and Drug Safety), 2015. The regulation on the safety standards of cosmetic and others. http://www.mfds.go.kr/index. Accessed 20 November 2016.

MFDS (Ministry of Food and Drug Safety), 2013. Esimation of external and internal dose for paraben risk assessment. Report No. 13162MFDS776. http://www.ndsl.kr/ndsl/search/detail/report/reportSearchResultDetail.do?cn=TRKO201400011747. Accessed 20 November 2016.

Moos RK, Angerer J, Dierkes G, Brüning T, Koch HM. 2015. Metabolism and elimination of methyl, *iso*- and *n*-butyl paraben in human urine after single oral dosage. Archives of Toxicology 90(11): 2699-2709.

Moreta C, Tena M-T, Kannan K. 2015. Analytical method for the determination and a survey of parabens and their derivatives in pharmaceuticals. Environmental Research 142: 452-460.

Oishi S. 2001. Effects of butyl paraben on the male reproductive system in mice. Archives of Toxicology 76: 423-429.

Oishi S. 2002. Effects of propyl paraben on the male reproductive system. Food and Chemical Toxicology 40: 1807-1813.

Pan S, Yuan C, Tagmount A, Rudel RA, Ackerman JM, Yaswen P, et al. 2016. Parabens and human epidermal growth factor receptor ligand cross-talk in breast cancer cells. Environmental Health Perspectives 124: 563-569.

Peng X, Xiong S, Ou W, Wang Z, Tan J, Jin J, et al. 2016. Persistence, temporal and spatial profiles of ultraviolet absorbents and phenolic personal care products in riverine and estuarine sediment of the Pearl River catchment, China. Journal of Hazardous Materials 323: 139-146.

Pop A, Drugan T, Gutleb AC, Lupu D, Cherfan J, Loghin F, et al. 2016. Individual and combined *in vitro* (anti) androgenic effects of certain food additives and cosmetic preservatives. Toxicology in Vitro 32: 269-277.

Prusakiewicz JJ, Harville HM, Zhang Y, Ackermann C, Voorman RL. 2007. Parabens inhibit human skin estrogen sulfotransferase activity: Possible link to paraben estrogenic effects. Toxicology 232: 248-256.

Pycke BF, Geer LA, Dalloul M, Abulafia O, Halden RU. 2015. Maternal and fetal exposure to parabens in a multiethnic urban US population. Environment International 84:193-200.

Rudel RA, Camann DE, Spengler JD, Korn LR, Brody JG. 2003. Phthalates, alkylphenols, pesticides, polybrominated diphenyl ethers, and other endocrine-disrupting compounds in indoor air and dust. Environmental Science & Technology 37: 4543-4553.

SCCS (Scientific Committee on Consumer Safety), 2011. Clarification on Opinion SCCS/1348/10 in the light of the Danish clause of safeguard banning the use of parabens in cosmetic products intended for children under three years of age.

http://ec.europa.eu/health/scientific_committees/consumer_safety/docs/sccs_o_069.pdf. Accessed 20 November 2016.

Soni M, Carabin I, Burdock G. 2005. Safety assessment of esters of p-hydroxybenzoic acid (parabens). Food and Chemical Toxicology 43: 985-1015.

Towers CV, Terry PD, Lewis D, Howard B, Chambers W, Armistead C, et al. 2015. Transplacental passage of antimicrobial paraben preservatives. Journal of Exposure Science and Environmental Epidemiology 25: 604-607.

Tran TM, Minh TB, Kumosani TA, Kannan K. 2016. Occurrence of phthalate diesters (phthalates), p-hydroxybenzoic acid esters (parabens), bisphenol a diglycidyl ether (BADGE) and their derivatives in indoor dust from vietnam: Implications for exposure. Chemosphere 144: 1553-1559.

Wang W, Kannan K. 2016. Fate of parabens and their metabolites in two wastewater treatment plants in New York state, United States. Environmental Science & Technology 50: 1174-1181.

Watanabe Y, Kojima H, Takeuchi S, Uramaru N, Ohta S, Kitamura S. 2013. Comparative study on transcriptional activity of 17 parabens mediated by estrogen receptor α and β and androgen receptor. Food and Chemical Toxicology 57: 227-234.

Ye X, Bishop AM, Reidy JA, Needham LL, Calafat AM. 2006. Parabens as urinary biomarkers of exposure in humans. Environmental Health Perspectives 114: 1843-1846.

Ye X, Bishop AM, Needham LL, Calafat AM. 2008. Automated on-line column-switching HPLC-MS/MS method with peak focusing for measuring parabens, triclosan, and other environmental phenols in human milk. Analytica Chimica Acta 622: 150-156.

Zhang N-S, Liu Y-s, Van den Brink PJ, Price OR, Ying G-G. 2015. Ecological risks of home and personal care products in the riverine environment of a rural region in South China without domestic wastewater treatment facilities. Ecotoxicology and Environmental Safety 122: 417-425.

Ⅶ. Supplementary information

Human pharmacokinetic profile of propyl paraben after oral administration

Table S1. LC-MSMS parameters for determination of parabens and the metabolites in serum and urine

Table S2. Intra-day and inter-day precision and accuracy of propyl paraben and the metabolites

Table S1. LC-MSMS parameters for determination of parabens and the metabolites in serum and urine.

Chemical	RT (min)	Precursor ion	Product ion	DP (volt)	EP (volt)	CE (volt)	CXP (volt)
n-propyl paraben-2,3,5,6-d_4 (PP-d_4)	6.9	183.1	96.0	-65	-10	-30	-5
p-hydroxybenzoic acid-2,3,5,6-d_4 (pHBA-d_4)	2.5	141.0	97.0	-45	-10	-16	-17
p-hydroxyhippuric acid (pHHA)	2.3	194.0	99.9	-40	-10	-14	-5
p-hydroxyhippuric acid-2,3,5,6-d_4 (pHHA-d_4)	2.3	197.8	99.9	-40	-10	-14	-5
$^{13}C_6$-methyl paraben ($^{13}C_6$-MP)	4.6	157.1	98.0	-75	-10	-30	-1
$^{13}C_6$-propyl paraben ($^{13}C_6$-PP)	6.9	185.0	97.9	-60	-10	-32	-17

RT: Retention time, DP: Declustering potential, EP: Entrance Potential, CE: Collision energy, CXP: Collision cell exit potential.

Table S2. Intra-day and inter-day precision and accuracy of propyl paraben and the metabolites.

Intra-day (N=15 for each chemical)

		Serum			Urine		
Chemical	Norminal concentration (μg/L)	Measured concentration[a] (μg/L)	RSD (%)	Accuracy (%)	Measured concentration[a] (μg/L)	RSD (%)	Accuracy (%)
PP-d_4	5	4.88 ± 0.4	7.5	97.6	4.81 ± 0.1	2.7	96.2
	20	20.4 ± 1.5	7.3	102.0	21.1 ± 1.1	5.0	105.4
	100	95.4 ± 6.4	6.7	95.4	104.8 ± 3.5	3.4	104.8
pHBA-d_4	5	5.71 ± 0.1	2.5	114.2	4.9 ± 0.17	3.5	98.0
	20	22.0 ± 1.2	5.6	110.0	19.7 ± 0.9	4.6	98.5
	100	104.9 ± 10.3	9.9	104.9	97.9 ± 2.9	2.9	97.9
pHHA	5	4.67 ± 0.2	4.6	89.4	5.04 ± 0.4	7.5	100.8
	20	19.3 ± 1.4	7.3	96.4	19.0 ± 1.2	6.2	95.0
	100	93.2 ± 8.9	9.5	93.2	87.0 ± 4.9	5.6	87.0

(Cont.)

Inter-day (N=45 for each chemical)

		Serum			Urine		
Chemical	Nominal concentration (μg/L)	Measured concentration[a] (μg/L)	RSD[b] (%)	Accuracy (%)	Measured concentration[a] (μg/L)	RSD[b] (%)	Accuracy (%)
PP-d_4	5	5.05 ± 0.4	7.1	101.0	4.88 ± 0.2	4.9	97.6
	20	20.4 ± 1.2	6.0	102.0	21.4 ± 1.5	7.2	107.0
	100	99.6 ± 5.8	5.8	99.6	106.0 ± 4.8	4.5	106.0
pHBA-d_4	5	5.33 ± 0.5	8.6	106.6	4.99 ± 0.3	6.2	99.8
	20	20.2 ± 1.9	9.7	100.9	20.0 ± 1.1	5.2	100.0
	100	100.6 ± 10.6	10.5	100.6	99.0 ± 5.2	5.3	99.0
pHHA	5	4.65 ± 0.5	9.9	93.0	5.05 ± 0.6	11.9	101.0
	20	19.9 ± 1.9	9.5	99.7	20.0 ± 2.7	13.4	100.1
	100	97.0 ± 10.3	10.6	97.0	98.8 ± 10.2	10.3	98.8

[a], The values were marked as AM ± SD; [b], RSD: Relative standard deviation

국문초록

경구 투여에 따른 프로필 파라벤의 인체 내 약물동태학적 프로파일 연구

서울대학교 보건대학원 환경보건학과

신 초 롱

파라벤(Paraben)은 개인위생용품 및 가정용품에 흔히 사용되는 살균성 보존제 성분 물질로, 최근 여러 연구들에 의해 내분비계 교란 영향 가능성이 제기되면서 그 안전성에 대한 논의가 이어지고 있다. 섭취에 따른 파라벤의 생물체 내 거동에 대한 연구는 동물을 대상으로 일부 수행된 바 있으나, 인체에 대한 자료는 부족한 실정이다. 따라서 본 연구에서는 프로필 파라벤과 대사산물의 약물동태학적 특성을 밝히기 위하여 건강한 성인 남성 5명을 대상으로 중수소 치환된 프로필 파라벤을 단위 체중 당 0.6 mg의 용량만큼 경구 투여하였으며, 노출 후 72시간까지 지정된 시점에 혈액과 소변을 수집하여 생체시료 내 프로필 파라벤과 가수분해 대사산물 2종(*p*-hydroxybenzoic acid and *p*-

hydroxyhippuric acid)의 농도를 측정하였다. 대사되지 않은 프로필 파라벤의 혈중 농도는 대사산물에 비해 현저히 낮은 것으로 나타났다. 또한 시간에 따른 혈중 농도의 프로파일에서 대사되지 않은 프로필 파라벤은 그 양상이 뚜렷하게 두 단계로 구분되었으나, 대사산물은 1차 반응을 따르는 것으로 확인되었다. 투여한 용량과 비교하였을 때 소변으로 배설되는 양은 총 프로필 파라벤 (대사되지 않은 프로필 파라벤과 중합체를 형성한 프로필 파라벤의 합을 의미)은 8.6%, 대사산물을 포함한 모든 물질에 대한 경우는 38.7%로 나타났다. 소변 중 가장 주된 대사체는 *p*-hydroxyhippuric acid였다. 혈중 농도 및 누적소변배출량 프로파일 자료를 바탕으로, 멀티 컴파트먼트 모델의 구조를 결정하고 이 모델을 이용해 약물동태학적 파라미터를 산출하였다. 그 결과 프로필 파라벤은 경구 투여 시 급속히 체내로 흡수되어 빠른 속도로 전신으로 분포되는 것을 확인할 수 있었다. 그러나 대부분의 프로필 파라벤은 가수분해 혹은 중합체 형성과 같은 대사 반응을 거친 뒤 소변을 통해 배설되었으며, 대사되지 않고 그대로 배설되는 것은 1% 미만으로 매우 적은 양이었다. 본 연구의 결과는 바이오모니터링을 이용한 한국인의 프로필 파라벤 노출 평가 시 활용될 수 있을 것이다.

주요어: 파라벤, 경구 노출, 약물동태학, 인체 대사, 누적 소변 배설 분율

학 번: 2015-24054