보건학석사학위논문

Pharmacokinetic features of benzophenone-3 after dermal application in humans

인체 경피 노출을 통한 벤조페논-3의 약물동태학 연구

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이 수 진

Abstract

Pharmacokinetic features of benzophenone-3 after dermal application in humans

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Benzophenone-3 (BP-3) is a component that blocks ultraviolet rays. It is mainly used in sunscreen agents and functional cosmetics, and is mostly exposed through the skin. several studies have reported the possibility of endocrine disturbance. Whereas pharmacokinetic studies on BP-3 have been reported in animal, it is not well known to the fate of BP-3 in human body following dermal exposure. The aim of this study is to determine pharmacokinetic characteristics of BP-3 in male subjects following single dermal application of 1 mg/cm^2 of deuterium labeled BP-3.

Blood and urine were collected for 72 hours and analyzed BP-3 and its metabolites using UPLC-MS/MS. In blood, after peak time eliminated decline two distinct phases in time-profile, while the metabolites followed first-order kinetics. Based on time-profiles, a multi-compartmental model constructed and validated. As a results, unconjugated BP-3 concentration in serum was lower than conjugated BP-3 concentration in serum. But it was similar to that of total BP-1 concentration in serum. Almost of the BP-3 and BP-1 in urine undergo conjugation or demethylation. And the fraction of urinary excretion for conjugated BP-3 (0.76 ± 0.19%) was 4-fold upper than total BP-1 (0.19 ± 0.13%), which was approximately 580-fold upper than unconjugated BP-3 (0.0013 ± 0.0005%). This study provides information on absorption, distribution, metabolism and elimination of BP-3 in human body and the pharmacokinetic model can be utilized for estimating exposure dose of BP-3, contributing to more realistic exposure assessment in the Korean population based upon biomonitoring data.

Keywords: bezophenone-3, dermal application, pharmacokinetic model, the fraction of urinary excretion, absorption · distribution · metabolism · excretion (ADME)

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Ⅰ. Introduction

The toxic potential of UV (ultra violet light) filters, used in a variety of sunscreen and personal care products to attenuate the negative effects of harmful UV radiation on skin and hair, has been a concern. Benzophenone-3 (BP-3, 2-hydroxy-4-methoxybenzophenone, oxybenzone) is one of the most widely used BP type UV filters and has been available as a sunscreen agent for over 40 years (Kim and Choi, 2014). It is found in over 1,500 products at concentrations up to 0.15% (EWG, 2016). In the U.S., BP-3 is a commonly used sunscreen agent (EWG, 2012). Because of the extensive use of BP-3 in personal care products, human exposure to this compound is widespread. BP-3 was found in >95% of urine samples collected from the U.S. general population (Calafat et al., 2008; Ye et al., 2005; Wolff et al., 2007), at concentrations ranging from 0.4 to 21700 ng/mL. The application of some personal care products that contain UV filters on the skin and frequent reapplication can increase the systemic absorption (Janjua et al., 2008; Jiang et al., 1999; León et al., 2010). BP-3 arose predominately after studies reported a systematic absorption of BP-3 in humans at a rate of up 1% to 2% after dermal application (Wang et al., 2011; Krause et al., 2012). Studies have linked endocrine-disrupting chemicals, which includes BP-3, with adverse birth outcomes via alterations in sex hormone activity during

development. BP-3 was shown to be weakly estrogenic in in vitro and in vivo (Fent et al., 2008; Schlumpf et al., 2001) and antiandrogenic in in vitro studies (Schreurs et al., 2005). Recent study also reported a significant association between exposure to BP-3 and an estrogen mediated disease, endometriosis in women (Kunisue et al., 2012).

BP-3 is rapidly absorbed from the intact skin and from gastrointestinal tract and demethylated and excreted in the urine in human (Wang et al., 2015). The metabolic pathways of BP-3 in human are shown in Fig. 1. BP-3 is metabolized to benzophenone-1 (BP-1, or 2,4-dihydroxybenzophenone) and benzophenone-8 (BP-8 or 2,2′OH-4MeO-BP or 2,2′-dihydroxy-4-methoxybenzophenone). BP-1 is formed via O-demethylation of the methoxy side chain on ring A of BP-3, whereas BP-8 is formed via the aromatic hydroxylation of ring B at the ortho position. A small portion of BP-1 can be further converted into THB via the aromatic hydroxylation of ring A at the meta position. It is noteworthy that the metabolite, BP-1, possesses greater estrogenic activity than does BP-3 (Kawamura et al., 2003, 2005; Nakagawa and Suzuki, 2002; Suzuki et al., 2005; Takatori et al., 2003).

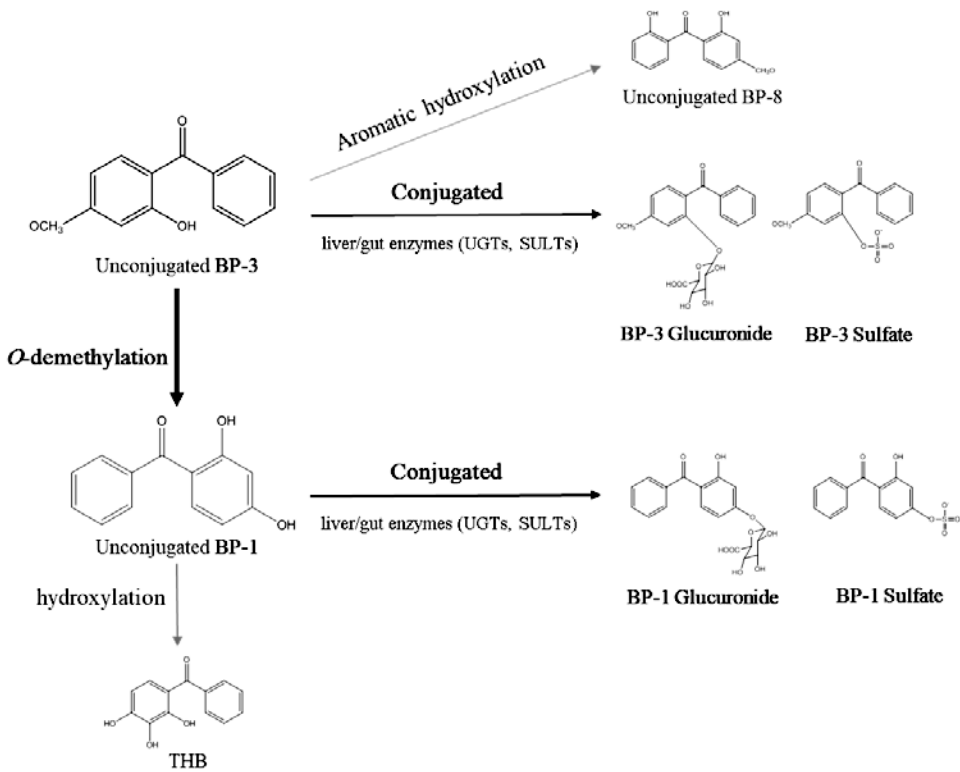


Figure 1. Metabolic pathways of benzophenone-3 in human. Adapted from Kim and choi (2014). Abbreviation: THB - 2,3,4-Trihydroxybenzophenone; SULTs - sulfotransferases; UGT - glucuronosyltransferases.

Several studies on the pharmacokinetics of BP-3 in animal have been reported (Jeon et al., 2008; Okereke et al., 1994), But research on human is quite limited. After topical application of BP-3 in female and male volunteers, systemic uptakes were confirmed (Gonzalez et al. 2002). And urinary excretion factor (F_{ue}) for BP-3 were reported. However, regarding to F_{ue}, it should be assumed that a simple steady-state kinetics for BP-3. Any kinetic information for BP-3 had been not suggested based on human data. Another studies investigated to BP-3 in blood, also any kinetic information for BP-3 and its metabolites had been not reported (janjua et al., 2008; tarazona et al., 2013).

Therefore, the elaborate study is required on the kinetics for BP-3 in humans. To the best of our knowledge, there are no pharmacokinetic study to simultaneously quantitate unconjugated and total (conjugated and unconjugated) BP-3 and its metabolites BP-1 in human urine and blood.

The objective of the study is to determine pharmacokinetic characteristics of BP-3 and its metabolite in volunteers after a single dermal application with a multi-compartment model. And then, it is possible to explain that how it was absorbed, distributed, metabolized and excreted in human body.

Ⅱ. Materials and methods

1. Chemicals and Reagents

2-Hydroxy-4-methoxybenzophenone-2',3',4',5',6'-d_5 (BP-3-d_5, 99%) and 2,4-Dihydroxybenzophenone-2',3',4',5',6'-d_5 (BP-1-d_5, 98.8%) were purchased from CDN Isotopes Inc. (Point-Claire, Quebec). $^{13}C_6$-2-Hydroxy-4-methoxybenzophenone ($^{13}C_6$-BP-3, 99%) was used as internal standard and obtained from Cambridge Isotope Laboratories Inc. (Tewksbury, MA). 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 (≥ 97%), β-Glucuronidase and sulfatase from Helix pomatia were purchased from Sigma-Aldrich (St. Louis, MO).

2. Study Design and Sample Collection

Five volunteers participated in the study. All participants were male and lived in Seoul, Korea. A single application with a cream containing 5% deuterated 2-Hydroxy-4-methoxybenzophenone (BP-3-d_5) to the right or left forearm for an hour. 200 mg for a forearm area of 200 cm^2 were applied to volunteers respectively. This dose is included in the usual range of thickness application for sunscreens (0.5–1 mg of cream per cm^2 of skin), which is usually below the recommended dose for a maximum sun protection 2 mg/cm^2 (KFDA, 2015). After an hour, the volunteers washed with a soap their arms. Blood and urine samples were monitored over 72 h and the first samples (T_0) before exposure were collected. The volume of each urine samples were measured and all samples were stored at -70 °C until analysis. The study was approved by the Seoul National University Institutional Review Board (SNUIRB # 1508/001-007). All participants were informed about the study design, and provided written informed consent in advance of the experiment.

3. Analytical procedure

3.1. Sample preparation

3.1.1 Blood sample preparation

The blood samples were prepared by the enzyme hydrolysis method as previously described (Lee et al., 2013; Dewalque et al., 2014; Ko et al., 2015) with a minor modification. In brief, 500 μL of an aliquot of serum was mixed with 10 μL of the internal standard solution $^{13}C_6$-BP-3, 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 BP-3 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 BP-3 in serum were determined following the same methodology described above without the enzymatic hydrolysis step.

3.1.2 Urine sample preparation

The urine samples were measured using an automatic, high throughput online SPE-UPLC-MS/MS method for measuring BP-3 in urine. Sample preparation was performed by following the method as previously described with some modifications (Ye et al., 2005, 2006). Briefly, 500 μL of an aliquot of urine was mixed with 10 μL of the internal standard solution $^{13}C_6$-BP-3, 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 100 μL of 0.1 M formic acid, and then centrifuged at 10000 rpm for 10 min. The supernatants were transferred to insert and confirm the bubble. Concentrations of unconjugated BP-3 in urine were determined following the same methodology described above without the enzymatic hydrolysis step.

3.2. UPLC-MS/MS analysis

Measurement of BP-3 and the metabolites in serum was performed by UPLC-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 compound specific MS/MS parameters are shown in the supplementary information (Table S1). 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 water (solvent A) and methanol (solvent B). Gradient condition was as follow; 0.0-2.5 min, 10% B; 2.5-3.5 min, 10%-100% B; 3.5-8.0 min, 100%-100% B; 8.6-13.0 min, 100%-10% min. The flow rate was 0.2 mL/min and 10 μL of each sample was injected into the UPLC system.

Measurement of BP-3 and the metabolites in urine was performed by on-line SPE-UPLC-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 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). The SPE column was an Oasis HLB column (2.1 × 20 mm, 5 um) from Waters, (Milford, MA). 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 trinary water (solvent A), methanol (solvent B), 10% methanol (solvent C). Gradient condition was as follow; 0.0-2.5 min, 10% B; 0-2.5min, 0.2 ml/min, C; Left valve, 0; 2.5-2.6min, 0 ml/min, C; 2.5-3.5 min, 100% B; 2.6-7.5min, 0 ml/min, C; 7.5-7.6min, 0.2 ml/min, C; 8.5 min, Left valve, 1; 3.5-8.5min, 100% B; 8.5-8.6min, 10%B; 8.6-13min, 100%-10%B. The flow rate was 0.2 mL/min and 10 μL of each sample was injected into the UPLC system. Quantitation was based on peak areas relative to the stable isotope-labeled internal standards. Because the dosing substance in this study was deuterium-labeled BP-3 (BP-3-d5) and BP-1 (BP-1-d5), we used $^{13}C_6$-labeled-BP-3 as an internal standard. Quality control measures were performed during every sample set, including

the 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 90.5% and 112.5% and the relative standard deviation (RSD) was 0.8% to 11.6%. The coefficient of variation for pooled-QC samples varied from varied from 0.3 % to 14.7 %. Calibration curve, ranging from 0.2 to 100 ng/mL, was plotted based on logarithmic ratio of the peak area of BP-3 and BP-1 to the peak area of the internal standard versus the logarithm of the BP-3 and BP-1 concentration (Yoon et al., 2015), whose regression coefficient (R) was >0.999 in urine, and (R) was >0.99 in serum. The limit of quantitation (LOQ) were 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 BP-3 and BP-1 were 0.2 ng/mL in both serum and urine.

4. Pharmacokinetic analysis

4.1. Non-compartmental analysis

Pharmacokinetic calculations were performed on each individual set of data using the pharmacokinetic calculation WinNonlin (Pharsight, St.Louis, MO, U.S.A.) by non-compartmental method. 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 BP-3 excreted in urine over 72 h divided by single dermal application dose.

4.2. Development of the pharmacokinetic model

As shown in Fig. 2, multi-compartment model was constructed to describe the pharmacokinetic properties of BP-3 in human after a single dermal application. The transdermal modeling was performed by permeation coefficient (k_p) obtained from the transdermal experiment and the skin-vehicle. and Fick's diffusion model was applied to percutaneous exposure (Corley, 2000; Brown et al., 1989). 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 five participants using Berkeley Madonna (University of California, Berkeley, CA, U.S.A.). The calibrated model was validated by evaluating the model prediction using urine data from other study.

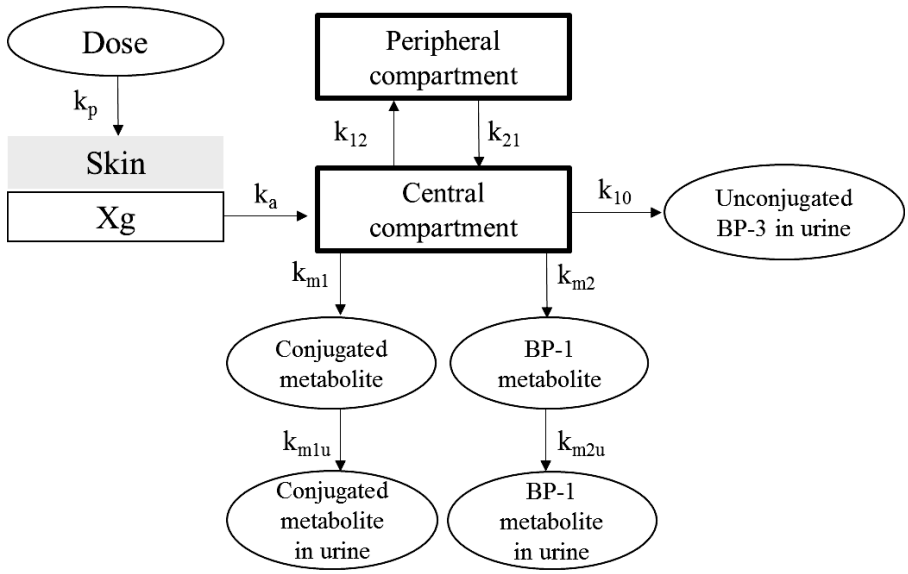


Figure 2. Structure of the pharmacokinetic model for BP-3 and its metabolites after dermal application. Abbreviations: k_p – The rate of absorption of BP-3 to skin (KFDA, 2016); k_a –absorbed skin dose of BP-3 to central compartment; k_{12} – BP-3 disposition from central compartment to peripheral compartment; k_{21} – reabsorption from peripheral compartment to central compartment; k_{10} – unconjugated BP-3 elimination at central compartment; k_{m1} – conjugated BP-3 formation in blood; k_{m1u} – conjugated BP-3 elimination in urine. k_{m2} – BP-1 formation in blood; k_{m2u} – BP-1 elimination in urine.

Ⅲ. Results

1. Participant characteristics

Five healthy males participated in the study, and demographic characteristics are presented in Table 1. The respectively average age, height, body weight and body mass index (BMI) of the subject were 24 years (range 20-28), 174 cm (range 163-182), 72 kg (range 62-89) and 24 (range 20-28).

Table 1. Demographic characteristics of the study participants.

Subject	Age	Height (cm)	Body weight (kg)	BMI (kg/m^2)
A	**25**	**182**	**67**	**20**
B	**28**	**167**	**62**	**22**
C	**26**	**163**	**72**	**27**
D	**20**	**177**	**89**	**28**
E	**20**	**181**	**71**	**22**
Mean ± SD	**24 ± 4**	**174 ± 9**	**72 ± 10**	**24 ± 4**

All participants were male (n=5).

2. Pharmacokinetic characteristics of benzophenone-3 and its metabolites

The semilogarithmic plot of time-concentration profile for BP-3 and its metabolites in serum is shown in Fig. 3. After a single application with a cream containing 5% BP-3, total BP-3, unconjugated BP-3 and total BP-1 present a similar profile. The serum concentrations of BP-3 and its metabolites increased 4 hours. From this point, BP-3 and its metabolites concentration decreased. The early phase with a rapid decline until 12 hours, and the second phase with a gradual and slow decline until 72 hours. Following these decline phase, the structure of the model was determined as a multi-compartment model.

Plots of serum concentrations of total, unconjugated BP-3 and total BP-1 at each time point following dermal application were analyzed using model-independent pharmacokinetic analysis. As shown in table 2, the mean T_{max} of unconjugated BP-3 (3.00 ± 0.71 h) was earlier than total BP-3 (3.20 ± 1.10) and total BP-1 (4.00 ± 2.35). The mean C_{max}, $AUC_{0-\infty}$, and $AUMC_{0-\infty}$ of unconjugated BP-3 were similar to total BP-1, whereas unconjugated BP-3 was about 2-fold lower than total BP-3. The terminal half-life ($T_{1/2}$) was calculated by dividing MRT_{last} by 1.44 (Boroujerdi, 2001). Parameter of unconjugated BP-3 was longer than total BP-3 and BP-1. CL_t, total body

clearance was estimated using an equation, $(CL_t)_{dermal}$ = F x D / AUC, where F is bioavailability and D is applied dose, bioavailability, the fraction of an applied dose that reaches the systemic circulation was assumed as 1.16% (SCCP, 2006). The total body clearance of BP-3 was 17.1 L/h.

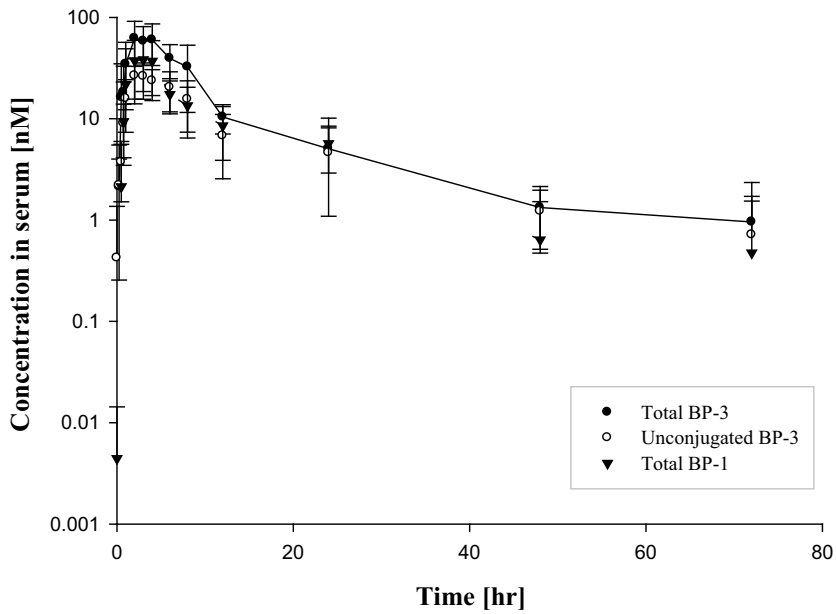


Figure 3. Time-concentration profile for BP-3 in serum. Each point and error bar represents the arithmetic mean and the standard deviation of volunteers (n=5). Concentrations below LOD were excluded.

Table 2. Pharmacokinetic parameters for benzophenone-3 in serum.

Parameter	Unconjugated BP-3	Total BP-3	Total BP-1
T_{max} (h)	3.00 ± 0.71	3.20 ± 1.10	4.00 ± 2.35
C_{max} (nM)	32.7 ± 7.53	70.9 ± 27.8	39.0 ±14.8
$AUC_{0-\infty}$ (nM·h)	336 ± 90.5	619 ± 156.3	361 ±76.9
$AUMC_{0-\infty}$ ($nM \cdot h^2$)	4730 ± 2744	7035 ± 3704	4913 ±2807
MRT_{last} (h)	13.0 ± 6.05	11.2 ± 5.91	12.9 ±5.69
$T_{1/2}$ (h)	9.06 ± 4.20	7.77 ± 4.11	8.93 ±3.95

Each value is revealed as arithmetic mean ± standard deviation of participants. T_{max}, peak time; C_{max}, peak concentration; $AUC_{0-\infty}$, area under the serum concentration-time curve between 0 and ∞; $AUMC_{0-\infty}$, area under first-moment curve between 0 and ∞; MRT_{last}, mean residence time; $T_{1/2}$, terminal half-life (n=5);.

Cumulative urinary excretion of BP-3 and its metabolites were presented in Fig. 4. BP-3 was mainly excreted as a metabolized form rather than unconjugated form. The fractional urinary excretion (F_{ue}, %) of total BP-3 was an average of 0.77% (range: 0.53~1.07%). The average F_{ue} of unconjugated BP-3 was 0.0013% (range: 0.0005~0.0019%), and the average F_{ue} of total BP-1 was 0.19% (range:0.09~0.41%).

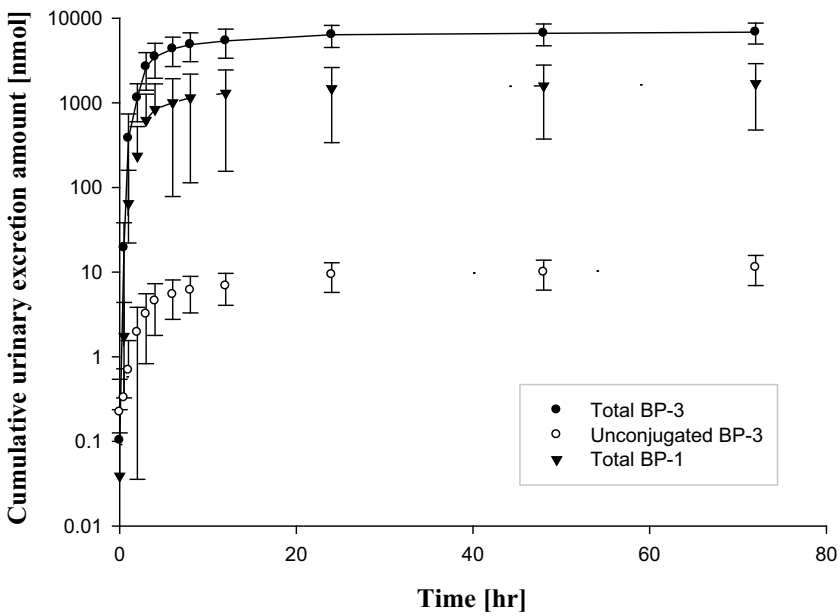


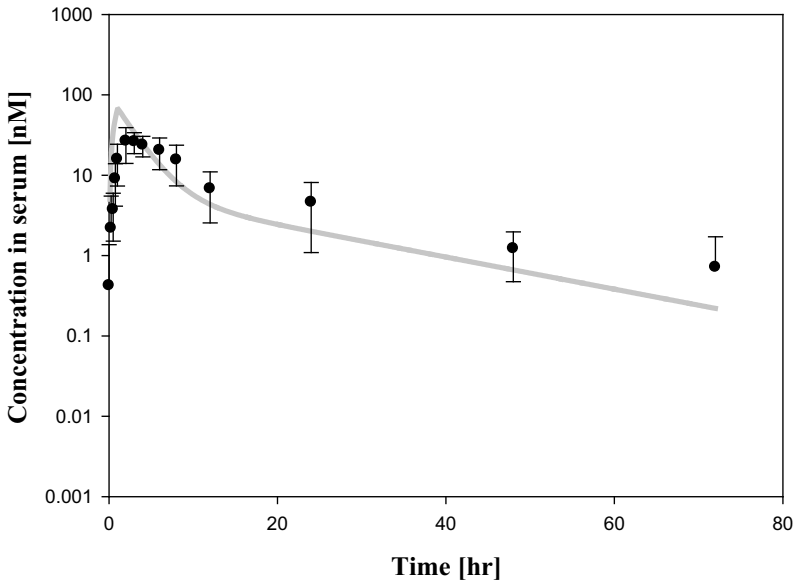
Figure 4. Accumulated amount of BP-3 recovered in urine. Each point and error bar represents the arithmetic mean and the standard deviation of volunteers (n=5). Concentrations below LOD were excluded.

3. Pharmacokinetic modeling

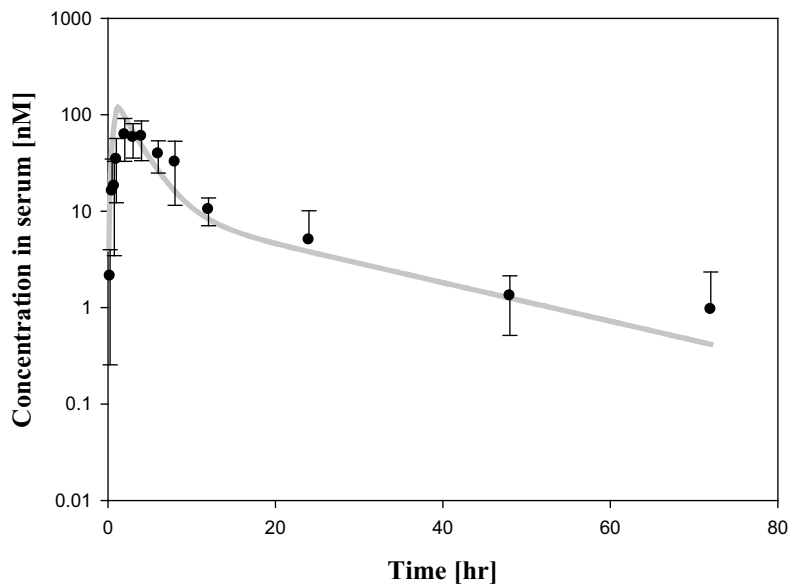
The multi-compartment model optimization for BP-3 and its metabolites was conducted (Fig. 5, 6). The model represented good prediction for time-concentration profile of BP-3 and its metabolites. Using this model, we determined the pharmacokinetic parameters, as shown in table 3.

The model was validated with observed urine data from Gonzalez et al., (2002) with a single dermal application of 2mg/cm^2 of BP-3 to seven males, four females (n=11). The kinetic parameters were fixed and applied dose, exposure body area, exposure duration were altered. The model validation is presented in Fig. 7.

(A) Unconjugated BP-3



(B) Total BP-3



(C) Total BP-1

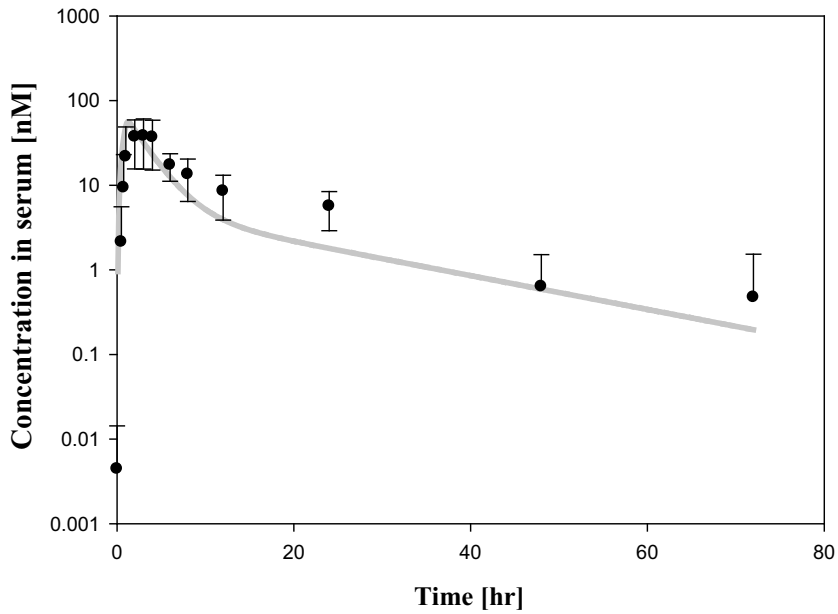
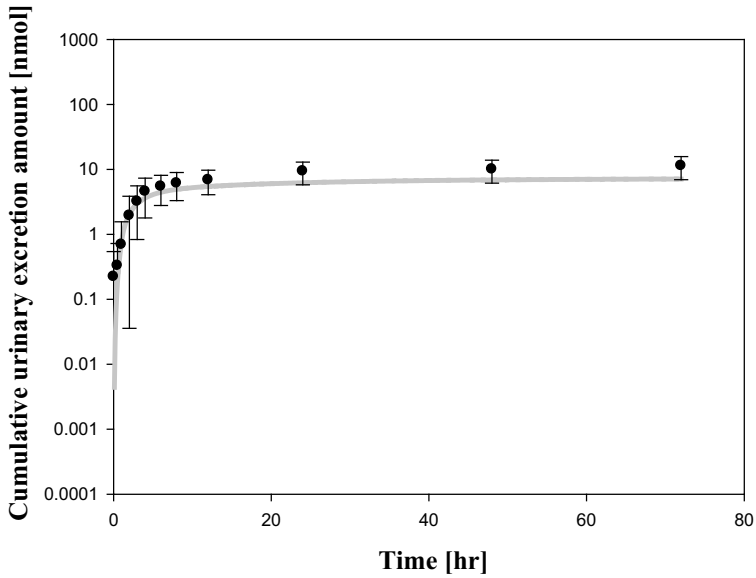
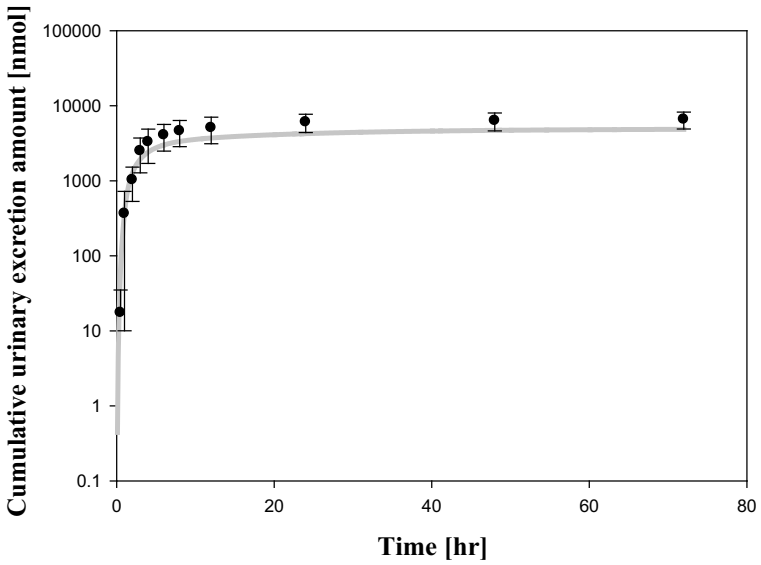


Figure 5. Model optimization using serum concentration. (A) unconjugated BP-3; (B) total BP-3; (C) total BP-1 in serum. Each point and error bar represent arithmetic mean ± standard deviation of participants. Solid line reveals multi-compartment model fit to the data. Concentrations below LOD were excluded.

(A) Unconjugated BP-3



(B) Total BP-3



(C) Total BP-1

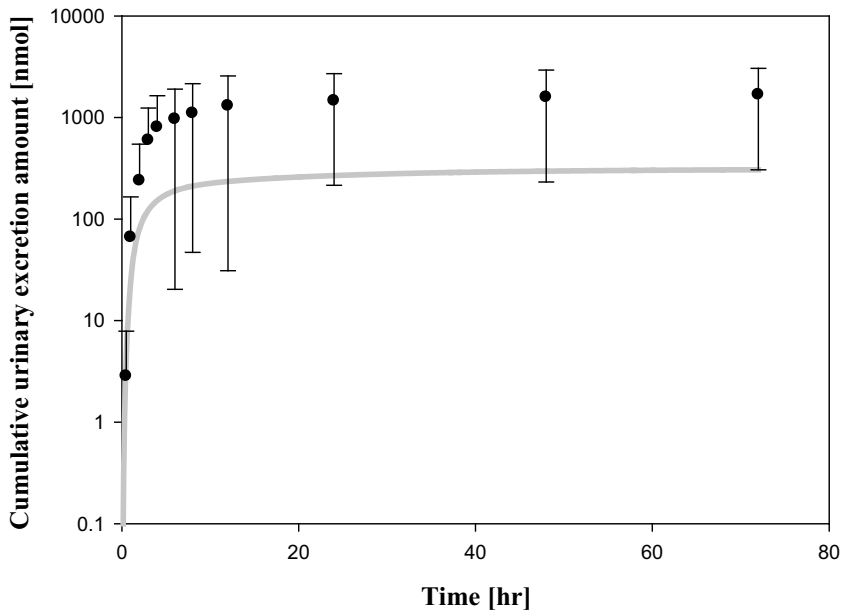


Figure 6. Model optimization using cumulative urinary excretion amount. (A) unconjugated BP-3; (B) total BP-3; (C) total BP-1 in urine. Each point and error bar represent arithmetic mean ± standard deviation of participants. Solid line reveals multi-compartment model fit to the data. Concentrations below LOD were excluded.

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

Parameter	Notes	Value
K_a, /h	Skin amount → Central compartment	0.39
K_{12}, /h	Central compartment → Peripheral compartment	8.67
K_{21}, /h	Peripheral compartment → Central compartment	0.07
K_{10}, /h	unconjugated BP-3 elimination from blood via urine	0.02
K_{m1}, /h	unconjugated BP-3 → Conjugated BP-3 formation	15.0
K_{m2}, /h	unconjugated BP-3 → Total BP-1 formation	0.95
K_{m1u}, /h	Conjugated BP-3 elimination from blood via urine	5.98
K_{m2u}, /h	Total BP-1 elimination from blood via urine	6.50
V_{pb}, L	Volume of distribution for unconjugated BP-3	1.02
V_{m1}, L	Volume of distribution for Conjugated BP-3	2.30
V_{m2}, L	Volume of distribution for Total BP-1	0.17
V_t, L	Volume of distribution for Total BP-3	1.90

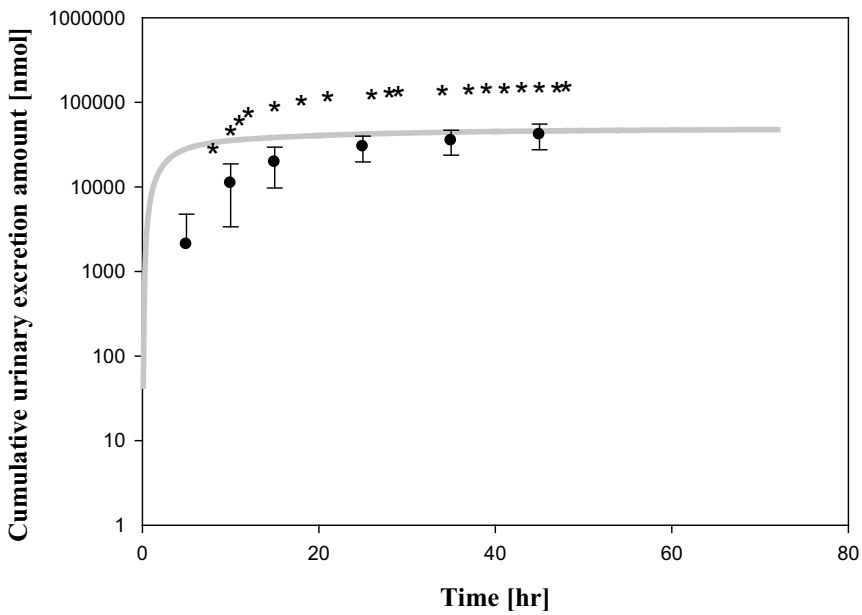


Figure 7. Model validation. Each point and error bar represent arithmetic mean and standard deviation of Gonzalez (2002)'s eleven volunteers (seven males and four females). Star symbols are maximum values of volunteer. Solid line represents multi-compartment model fit to the data.

Ⅳ. Discussion

Benzophenone-3 (BP-3) is a chemical blocking agent that blocks ultraviolet light. It is mainly used in sunscreen agents and functional cosmetics and is mostly exposed to the skin. Several studies have reported the possibility of endocrine disturbance, while pharmacokinetic data or model for BP-3 in humans were limited. For this reason, we represented the skin using a more empirical compartment model. In the present study, we investigated the time profiles of BP-3 and its metabolites in serum and urine after dermal application 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 BP-3 following dermal exposure.

BP-3 is widely used in commercial sun protection products at a maximum concentration of 5% for BP-3 and the maximum formulation standard of BP-3 is regulated below 2 mg/cm^2 (KFDA, 2015). A number of studies have shown that consumers apply much less than this typically between 0.5 and 1.5 mg/cm^2 (Diffey et al., 2001). We considered the legal standards, the thickness, amount of the area and formulation of sunscreen that people commonly use. In this study, cream (200 mg) of containing 5% BP-3 were exposed to the forearm (200 cm^2) for 1 hour. After dermal application of

BP-3, they may be absorbed and accumulated in the body. They pass through the skin and reach the kidneys, where they are metabolized and further excreted. Urine is the primary route, with feces being the secondary route for elimination of both parent compounds and metabolites. Toxicity studies on human plasma and urine samples revealed *o*-dealkylation of the methoxy side chain to be the major pathway of BP3 metabolism. This mechanism yields the most abundant metabolite, 2,4-dihydroxybenzophenone (BP-1). Aromatic hydroxylation is a secondary pathway, in which metabolites 2,3,4-trihydroxybenzophenone (THB) and 2,2-dihydroxy-4-methoxy-benzophenone (BP-8) are formed (Díaz-Cruz et al., 2008). And BP-8 was detected in trace amounts in urine samples, but copious amounts were detected in fecal samples (Okereke et al., 1994). In the case of BP-3, studies in rats have reported substantial dermal metabolism, protein binding and excretion in both urine (67%) and feces (21%) with other studies reporting excretion in breast milk (Jiang et al., 1999). In order to investigate of BP-3 pharmacokinetic characters, except BP-8 which is mainly detected in feces, this study analysis BP-3 BP-1 and THB in bio-samples. However, in the case of THB, THB could not be analyzed as the analysis method of this study. Therefore, only BP-3 and BP-1 were measured in this study.

The semilogarithmic plot of time-concentration profile in serum (fig.3) demonstrated that the pharmacokinetics of BP-3 could be explained by a multi-compartment model. The volume of distribution for central

compartment (V_{pb}) was 1.02 L, for conjugated BP-3 was 2.30, for total BP-1 was 0.17 L and that for total BP-3 was 1.90 L. Consist of the model rate constants were assumed to follow first-order kinetics. After a single application with a cream containing 5% BP-3, BP-3 was permeated to skin using k_p and then BP-3 in skin was distributed to central compartment (k_a=0.39). Unconjugated BP-3 in central compartment was rapidly distributed to peripheral compartment (k_{12}=8.672), whereas slowly redistributed to central compartment (k_{21}=0.071). Unconjugated BP-3 was eliminated in urine (k_{10}=0.02). Unconjugated BP-3 was conjugated such as BP-3-glucuronide and BP-3-surfate (k_{m1}=15.03), which were rapidly excreted in urine because of increased in water solubility (k_{m1u}=5.98). And Unconjugated BP-3 was metabolized into BP-1 (k_{m2}=0.95), which were rapidly excreted in urine (k_{m2u}=6.50).

Cumulative urinary excretion of total BP-3 as shown in Fig. 4 provided that an average of 0.77% of applied BP-3 was excreted in urine. Gonzalez (2002) had previously performed similar work to this study. Containing 4% BP-3 lotion applied to 11 participants (7 males, 4 females). They applied it over the whole body (except for the scalp and genital areas). And participants put on their normal clothes after the lotion was absorbed to the skin. And then, they could shower only once, after 12 hours during 48 hours period. The average total amount excreted in urine approximately 0.5% of the applied amount of BP-3. To validated the our PK model, we used to Gonzalez et al.,

(2002) recoverd amount data of BP-3 in urine. As shown in Fig. 7., Model fitness results show that cumulative urine data of BP-3 was fitted within standard deviation and maximum observed data. But early preliminary time point didn't fit well. Gonzalez data shown that BP-3 absorption was delayed as like lag time. Lag time that is a function of the drug loading the stratum corneum and dermis, diffusivity, and thickness of the skin (Lazaridis et al., 2010). The reason is that previous study and this study were used dermal application of different type such as lotion versus cream. And race also different. Another study is that Hayden, Roberts and Benson reported on the systemic absorption in humans of BP-3 after topical application. Analysis of the urine using β-glucuronidase suggests that BP-3 and its metabolites undergo extensive conjugation in the body. It is estimated that the actual amount absorbed from the applied formulation over 10 hours period was between 1 and 2% of the applied amount contained in the product. This study also performed analysis of the urine using β-glucuronidase plus sulfatase. In order to excrete in urine, Almost of the BP-3 and BP-1 in urine undergo conjugation or demethylation. And the fraction of urinary excretion for conjugated BP-3 (0.76%) was 4-fold upper than total BP-1 (0.19%), which was approximately 580-fold upper than unconjugated BP-3 (0.0013%).

One of limitation of the study is that we don't know how much cream of BP-3 are absorbed. Therefore, we estimated how much was absorbed into

the skin with oral CL in serum (KFDA, 2015). According to $\frac{CL_{dermal}}{CL_{oral}}$, it is absorbed by the skin to about 5 ± 1% of the absorption by ingestion. Another limitation of the study is that study population. The pharmacokinetic model for BP-3 is constructed based on a specific population as healthy male. And the model validation was only performed using the data from Gonzalez et al., (2002). Additional model validation processes using data in blood are needed in order to verify universal applicability of the model. And pharmacokinetic model in the study can't take account of all situation about using sun screen. We performed to dermal application during only 1 hour and single exposure, whereas people usually applied it all day or more than eight hours. And some people apply it several times a day. In spite of the limitations, a quantitative knowledge on human pharmacokinetic of BP-3 is essential to investigate BP-3 exposure using biomonitoring data. Simple pharmacokinetic model is used to reverse dosimetry by biomonitoring data and evaluate risk assessment. In risk assessment, using pharmacokinetic model instead of estimated equation might reduce uncertainties (US EPA, 2002).

Ⅴ. Conclusions

In conclusion, the present study determined the time-concentration profiles of BP-3 in human serum and urine after a single dermal application, and estimated pharmacokinetic parameters through non-compartmental analysis. Furthermore, it was found that the multi-compartment model constructed based on the profiles well described the pharmacokinetic characteristics of BP-3. This study provides pharmacokinetic data of BP-3, which help to understand the absorption, distribution, metabolism and excretion of BP-3 in human body after topical application. Moreover, despite the need for further validation in serum process, the human pharmacokinetic model for BP-3 can be a useful tool for reconstructing exposure dose of BP-3 by applying human biomonitoring data, ultimately contributing to more realistic exposure assessment.

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Ⅶ. Supplementary information

Pharmacokinetic features of benzophenone-3 after dermal application in humans

Table S1. LC-MSMS parameters for determination of benzophenone-3 and the metabolites in serum and urine

Table S 1. LC-MSMS parameters for determination of benzophenone-3 and the metabolites in serum and urine.

Chemical	RT (min)	Precursor ion	Product ion	DP (volt)	EP (volt)	CE (volt)	CXP (volt)
2-Hydroxy-4-methoxybenzophenone-2',3',4',5',6'-d5 (BP-3-d_5)	6.48	232.1	214.7	-60	-10	-30	-15
2,4-Dihydroxybenzophenone-2',3',4',5',6'-d5 (BP-1-d_5)	6.1	218	134.8	-70	-10	-28	-9
$^{13}C_6$-2-Hydroxy-4-methoxybenzophenone (BP-3-$^{13}C_6$)	6.49	233.1	216.8	-70	-10	-28	-15

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

국문초록

인체 경피 노출을 통한 벤조페논-3의 약물동태학 연구

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이 수 진

벤조페논-3 (BP-3)는 자외선을 차단하는 성분으로 자외선 차단제, 기능성 화장품에 주로 사용되고, 대부분 피부를 통해 노출이 된다. 최근 여러 연구들에 의해 내분비계 교란 영향 가능성이 제기되면서 그 안전성에 대한 논의가 이어지고 있다. 피부 노출에 따른 BP-3의 생물체 내 거동에 대한 연구는 동물을 대상으로 일부 수행된 바 있으나, 인체에 대한 자료는 부족한 실정이다. 본 연구의 목적은 BP-3의 단회 경피 노출 후 인체 내에서의 흡수, 분포, 대사 및 배설에 대한 특성을 파악하고, 약물동태학적 모델을 구축하여 이를 설명하는 것이다. 이를 위해 건강한 성인 5명을 대상으로 식품의약품안전처의 화장품 배합 기준인 5 %로 제조한 중수소 치환된 BP-3 크림 1 mg/cm^2 를 한

쪽 팔 전완 부분 안쪽 (200 cm^2)에 도포하여, 1시간 동안 노출시켰다. 노출 전 그리고 노출 후 72시간까지 지정된 시점에 혈액과 소변을 수집하였으며, UPLC-MS/MS 를 이용하여 생체시료 내 BP-3와 대사산물의 농도를 측정하였다. 혈중 프로파일 최대값에서 시간에 따라 혈중 농도가 감소하는 양상이 뚜렷하게 두 단계로 구분되는 것을 확인하였고, 이를 바탕으로 다중-컴파트먼트 모델을 구축하고 검증하였다. 대사 되지 않은 BP-3의 혈중 농도는 BP-3 중합 반응 대사 산물에 비해 낮으나, 탈 메틸화를 거친 BP-3 대사산물과 비슷한 수준으로 나타났다. 피부에 도포한 용량과 비교하였을 때 소변으로 배설되는 양은 총 BP-3는 0.77 ± 0.19%, 대사산물인 총 BP-1 경우는 0.19 ± 0.13%로 나타났다. 이 연구에서 BP-3는 경피 노출 시 체내로 흡수되어 빠른 속도로 전신으로 분포되는 것을 확인할 수 있었다. 그리고 대부분의 BP-3는 탈 메틸화 혹은 중합체 형성과 같은 대사 반응을 거친 뒤 소변을 통해 배설되었으며, 중합체 형성과 같은 대사 반응을 하지 않은 BP-3는 0.0013 ± 0.0005% 매우 적은 양으로 배설되었다. 본 연구는 인체 내에서 BP-3의 흡수, 분포, 대사 및 배설에 관한 정보를 제공하며 구축된 약물동태학적 모델은 바이오모니터링을 이용한 한국인의 BP-3 경피 노출 용량을 역산하는데 활용함으로써 보다 정확한 노출 평가에 기여할 수 있을 것이다.

주요어: 벤조페논-3, 경피 노출, 약물동태학 모델, 누적 소변 배출량, 흡수 · 분포 · 대사 · 배설

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