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# Prediction of first-in-human dose for new composition bee venom based on allometric scaling and pharmacokinetic modeling approach

**Soon Uk Chae** [1,†], **Jee Sun Min** [1,†], **Seong Jun Jo** [1,2], **Chae Bin Lee** [3], **Jinha Park** [4], **Soo Hyeon Bae** [4,*], **and Soo Kyung Bae** [1,*]

[1]College of Pharmacy and Integrated Research Institute of Pharmaceutical Sciences, The Catholic University of Korea, Bucheon 14662, Korea
[2]Department of Pharmaceutical Sciences, State University of New York, Buffalo, NY 14214, USA
[3]Johns Hopkins Drug Discovery, Department of Neurology, Johns Hopkins School of Medicine, Baltimore, MD 21205, USA
[4]AIMS BioScience, Seoul 06591, Korea



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***Correspondence to**
**Soo Hyeon Bae**
AIMS BioScience, 222 Banpo-daero, Seocho-gu, Seoul 06591, Korea.
Email: sh.bae@aimsbiosci.com

**Soo Kyung Bae**
College of Pharmacy and Integrated Research Institute of Pharmaceutical Sciences, The Catholic University of Korea, 43 Jibong-ro, Wonmi-gu, Bucheon 14662, Korea.
Email: baesk@catholic.ac.kr

[†]Soon Uk Chae and Jee Sun Min equally contributed to this work.



**ORCID iDs**
Soon Uk Chae
https://orcid.org/0000-0002-4367-9004
Jee Sun Min
https://orcid.org/0000-0002-0909-116X
Seong Jun Jo
https://orcid.org/0009-0002-6838-2861
Chae Bin Lee
https://orcid.org/0000-0003-4551-9191

## ABSTRACT

Bee venom is a traditional remedy used to treat conditions related to the nervous and musculoskeletal systems, as well as autoimmune diseases. Recently, we developed a new composition bee venom (NCBV), a fortified content of bee venom phospholipase A2 (bvPLA2), which may be effective in the treatment of Alzheimer's disease. NCBV is currently preparing to conduct a phase 1 clinical trial, and this study aimed to predict the first-in-human (FIH) dose using a mechanistic approach. First, animal pharmacokinetic (PK) studies from three different species were explored and integrated to build a PK model using nonlinear mixed-effect modeling. The final models were described by two-compartment model with first order absorption and elimination, and were used to define the PK parameters for each species. To predict human PK parameters, simple, brain weight (BrW) or maximum lifespan potential (MLP) incorporated allometric scaling approaches were used, with the BrW method showing the highest correlation ($R^2$ = 0.974). The initial FIH dose was back-calculated based on the area under the concentration-time curve of 0.397 μg·h/mL after the injection of an efficacious dose of 0.1 mg/kg in mice using the developed PK model. The predicted initial doses for a 70 kg human were 5.5, 1.3, and 3.5 mg, when using the simple, BrW, and MLP incorporated model, respectively. A subcutaneous FIH dose of 1.3 mg NCBV was ultimately recommended for a 70-kg human. Based on the no observed adverse effect level, the suggested FIH dose ranges for NCBV are 0.1 to 3 mg, which correspond with our proposed dose.

**Keywords:** Bee Venom Phospholipase A2; Pharmacokinetics; Nonlinear Mixed-Effect Modeling; Allometric Scaling; FIH Dose

## INTRODUCTION

Estimating the first-in-human (FIH) dose plays a crucial role in drug development during phase 1 clinical trials, ultimately paving the way for the Food and Drug Administration (FDA) approval [1]. To estimate initial dose for humans, accurate prediction of pharmacokinetic

Jinha Park
https://orcid.org/0009-0005-7903-8793
Soo Hyeon Bae
https://orcid.org/0000-0001-5896-5938
Soo Kyung Bae
https://orcid.org/0000-0001-7258-7187

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**Conflict of Interest**

- Authors: Nothing to declare
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**Author Contributions**

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(PK) parameters in humans is essential [2]. There are two main approaches for predicting PK parameters in humans using data from animal studies: allometric scaling and physiologically based pharmacokinetic (PBPK) modeling. Allometric scaling is a well-established and widely accepted empirical method for extrapolating PK parameters across species [1-5]. In contrast, PBPK modeling provides a mechanistic solution for extrapolating data from one species to another. PBPK models enable the incorporation of greater complexities but typically require substantial resources and time for development [6]. Additionally, the calculations involved in allometric scaling are relatively simple [7]. Hence, interspecies allometric scaling is mainly used to extrapolate PK parameters from animals to human during drug development [1]. Allometric scaling primarily focuses on the interaction between clearance (CL), and volume of distribution ($V_d$) of unbound drugs and the body weight of different species. These parameter relationships, initially established in preclinical species, are subsequently extrapolated to humans, enabling the prediction of CL and $V_d$ in humans [8].

However, individual animal data from preclinical PK studies often exhibit inconsistencies and gaps. To address this issue, a single-step approach using nonlinear mixed-effect modeling (NONMEM) pools individual data from all animals, enabling simultaneous scaling of PK parameters across species and facilitating the simulation of various dosing scenarios for the FIH study [9].

Moreover, predicting the FIH dose is challenging due to its inherent uncertainty and should be supported by nonclinical safety and PK data [2]. Therefore, various methods have been developed to predict doses for humans; both empirical and mechanistic approaches are frequently applied [10]. Among these, the empirical method is the most widely used approach, in accordance with the FDA guidelines [11]. The approach predicts FIH dose by converting the no observed adverse effect level (NOAEL) to a dose normalized of to body surface area using allometric scaling and a safety factor [11]. However, a limitation of this approach is that the calculation of the dose solely on safety, while the efficacy of the drug is not considered a decision factor for the FIH dose. On the other hand, the mechanistic approach selects a dose that is anticipated to have minimal biological effects, including the dose predicted to be safe based on the NOAEL, in accordance with European Medicines Agency guidelines [12]. Therefore, the recommended dose is expected to be both pharmacologically effective and safe [10].

Bee venom, a toxin secreted by honeybees (*Apis mellifera*), contains a variety of peptides, such as melittin (50–60%), apamin (1–3%), mast cell degranulating peptide (1–3%), and adolapin (0.1–0.8%). Among these, dried bee venom typically consists of approximately 10–12% bee venom phospholipase A2 (bvPLA2) [13]. Leveraging the established efficacy of bee venom and bvPLA2 in Alzheimer's disease, a new composition bee venom (NCBV) was developed with an increased bvPLA2 concentration of up to 76.2%. NCBV was derived from crude honeybee venom and lyophilized using a 10 kDa ultrafiltration manufacturing process by the INISTst R&D Center [14]. Although the PK profile of bvPLA2 in rats was previously determined [15], this data provided limited information for the early clinical development of this drug. Therefore, in the present study, we obtained PK profiles of bvPLA2 from mice, rats, and beagle dogs following the subcutaneous (SC) injection of NCBV to build the models that predict the changes in plasma concentrations of bvPLA2 over time and PK parameters. Subsequently, the parameters were used for allometric scaling to predict PK parameters in human. Based on this information, initial SC dose for humans in phase I clinical trials was estimated using the mechanistic approach.

# METHODS

## Overall strategy

Overall human PK and FIH dose estimation proceeded as follows. First, animal PK studies from three different species were conducted to capture animal PK profiles. Second, the collected animal PK data were integrated to build the compartment model. Third, the developed animal PK models were used to define PK parameters for each species. Fourth, human PK parameters were predicted using an allometric scaling based on the parameters derived from the developed animal PK model. Fifth, the predicted human PK parameters were used to simulate PK profiles of human at pharmacologically effective level and SC administration dose for the FIH study was estimated. Finally, the predicted FIH dose was compared to the FIH dose derived from the NOAEL.

## Animals

Institute of Cancer Research (ICR) mice (7–8 weeks old, 20–35 g) and male and female Sprague-Dawley (SD) rats (7–9 weeks old, 200–330 g) were purchased from Young Bio (Seongnam, Korea). The animal study protocol was approved by the Institutional Animal Care and Use Committee of Department of Laboratory Animals, the Catholic University of Korea, Songsim campus (approval No. 2019–040). The rats and mice were housed under controlled environmental conditions (temperature 20 ± 2°C; relative humidity 55 ± 5%; 12/12-h light/dark cycle). The study on beagle dogs was approved by the ChemOn Laboratory Animal Management Committee (approval No. 20-D283).

## SC injection of NCBV in mice, rats, and beagle dogs

Single SC injection of NCBV dissolved in normal saline was administered to male and female ICR mice (0.5 mg/kg), male and female SD rats (0.2, 0.5, 1, and 2 mg/kg), and beagle dogs (0.3, 0.6, and 0.9 mg/kg). Owing to the limited blood volume that can be collected from mice, the parallel cardiac puncture method was used to collect blood samples (1 mL). Rat blood samples were obtained from the carotid artery (200 μL), and beagle dog blood samples were obtained from the cephalic vein (2 mL). Blood samples were collected at 0 (pre-dose), 0.05, 0.08, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, 8, 10, and 24 hours following the SC injection of NCBV in mice (n = 4 at each time point; total n = 48 per sex) and rats (n = 4 per sex per dose group) and at 0 (pre-dose), 0.25, 0.5, 1, 2, 4, 8, and 24 hours in beagle dogs (n = 4 per sex per dose group). After centrifugation at 14,000 rpm for 10 minutes, the plasma was separated and stored at −20°C. All beagle dog experiments were conducted by ChemOn (Yongin, Korea).

## bvPLA2 analysis in the plasma

Whole plasma samples were kept at −70°C until further analysis. Enzyme-linked immunosorbent assay (ELISA) was performed using the bvPLA2 ELISA kit purchased from AbClon Inc. (Seoul, Korea). The plasma concentration of bvPLA2 was determined using a previously described analytical method [15]. The calibration curve spanned from 0.78 to 100 ng/mL. Using a weighting factor of $1/C^2$, a 4-parameter logistic calibration curve was implemented in SoftMax Pro 6.x software to interpolate the concentrations of bvPLA2. bvPLA2 analysis in beagle dog plasma samples was performed by ChemOn. The correlation coefficient ($R^2$) of the standard curves were greater than 0.99, and accuracy and precision of all QC samples were within 15% of the nominal concentration.


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### Non-compartmental analysis of bvPLA2 in mice, rats, and beagle dogs

Phoenix® WinNonlin® software (version 6.0; Certara USA, Princeton, NJ, USA) was used to conduct a non-compartmental analysis and calculate the basic PK parameters.

### PK model development

The development of the PK model was conducted using the ADVAN4 and TRANS4 subroutines and the first-order conditional estimation with interaction (FOCE-I) methods of NONMEM (version 7.5; ICON Development Solutions, Ellicott City, MD, USA) running AcroEdit® (version 0.9; AcroSoft, Korea). The plasma concentration-time profiles of bvPLA2 following NCBV injections were described using a two-compartment model with first-order absorption and elimination. Elimination of bvPLA2 was assumed to occur only in the central compartment. The inter-individual variabilities (IIVs) of PK parameters were described using a log-normal distribution of the structural model parameters:

$$P_i = \theta \times exp(\eta_i)$$

where $P_i$ is the parameter for the individual i, θ is the typical population value of the parameter, and $\eta_i$ is the inter-individual random effect with a mean of 0 and variance of $\omega^2$.

The residual variability of the bvPLA2 parameter was computed using a modified model that combined additive and proportional error components and is expressed as follows:

$$\omega_{ij} = \sqrt{{\sigma_{add}}^2 + {\sigma_{pro}}^2 \times \mathrm{IPRED}^2}$$

$$Y_{ij} = \mathrm{IPRED} + \omega_{ij} \times \varepsilon_{ij}$$

where $Y_{ij}$ represents the observed dependent variable for the $i^{th}$ individual at time j, IPRED is the individual predicted value, $\omega_{ij}$ represents the residual standard deviation and is often used to account for the variability or uncertainty in the observed data, and $\varepsilon_{ij}$ represents a residual error term, often referred to as the model's residuals or errors.

Model selection was based on several criteria including the plausibility and precision of parameter estimates, goodness-of-fit (GOF) plots, individual prediction plots, decrement in the objective function value (OFV), eta shrinkage, and successful convergence. The final models were selected based on their best fit to the data and their ability to accurately describe the observed data.

### Model evaluation

The model's ability to predict PK was evaluated using a visual predictive check (VPC) that encompassed 1,000 virtual datasets simulated using the final model [16,17]. The VPC was performed using NONMEM, and the results were visualized using Xpose from the R statistics package (version 4.3.1) [18]. The median and nonparametric 90% prediction intervals, including the $5^{th}$ to $95^{th}$ percentiles, were computed using simulated plasma concentrations to assess the final model. The observed concentration data were visually compared with the median values and $5^{th}$–$95^{th}$ percentiles obtained from the simulated concentration-time profiles. Comparisons were made to evaluate the performance of the model.


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### Allometric scaling and prediction of human PK parameters

The allometric equation allows the prediction of PK parameters in any animal species using the product of an allometric coefficient and body weight raised to a power function. The general allometric power model, which is a simple model, is represented mathematically by the following equation:

$$Y = a \times BW^b$$

Where *Y* is PK parameters (i.e., CL or $V_d$) and a and b are the coefficient and exponent of the allometric equation, respectively [19-21]. This allometric relationship can be represented as a linear relationship when plotted using logarithmic coordinates (log-log scale). Where log(a) is the y-intercept and b is the slope of the curve. The CL, central volume of distribution ($V_c$), peripheral volume of distribution ($V_p$), and absorption rate constant ($k_a$) estimated using compartmental analysis in mice, rats, and beagle dogs were used to predict the corresponding parameters in humans. A linear relationship was established by fitting the log-transformed data to estimate the parameters "a" and "b."

To improve the correlations, two additional approaches considering physiological factors, either brain weight (BrW) or maximum lifespan potential (MLP), were also projected and used to define allometric relationship as described previously [5]. The final equations derived using each method were compared to others and applied to predict PK parameters in humans, respectively.

### Human PK simulation and estimation of FIH dose

The three different sets of PK parameters predicted in humans were applied to the model independently, and the plasma concentration-time profile of bvPLA2 following a single SC injection of NCBV for 24 hours was simulated. The dose of NCBV for the human PK simulations was determined considering the efficacious exposures in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-induced mouse models [22]. It is assumed that the level of efficacious exposures correlated with area under the curve (AUC), and the FIH dose was back-calculated based on the AUC at the efficacious dose derived from the developed mouse PK model. The final FIH dose estimation was determined by selecting the method that showed the highest coefficient of correlation ($R^2$ value) among the three allometric scaling approaches. Then, the predicted FIH dose was compared to the suggested FIH dose range for NCBV based on the toxicological profile and NOAEL.

## RESULTS

### Non-compartmental analysis of bvPLA2 in mice, rats, and beagle dogs

The mean bvPLA2 PK parameters of bvPLA2 in mice after a single SC injection of 0.5 mg/kg NCBV is listed in **Table 1**. The peak plasma concentration ($C_{max}$) was 475 ng/mL at 0.500 hours and 474 ng/mL at 0.375 hours in male and female mice, respectively. The half-life ($t_{1/2}$) was approximately 2.73 and 1.75 h in male and female mice, respectively. The area under the concentration-time curve ($AUC_{0-t}$) was 1.38 and 1.19 μg·h/mL in male and female mice, respectively.


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**Table 1.** Pharmacokinetic parameters of bvPLA2 after single subcutaneous injection of NCBV to mice, rats and beagle dogs, respectively

| Parameters | Mice | Rats | | | | Beagle dogs | | |
|---|---|---|---|---|---|---|---|---|
| | 0.5 mg/kg | 0.2 mg/kg | 0.5 mg/kg | 1 mg/kg | 2 mg/kg | 0.3 mg/kg | 0.6 mg/kg | 0.9 mg/kg |
| $AUC_{0-t}$ (μg·h/mL) | 1.28 | 0.101 ± 0.0678 | 0.209 ± 0.131 | 0.586 ± 0.203 | 1.22 ± 0.251 | 1.35 ± 0.432 | 2.29 ± 0.577 | 3.15 ± 0.474 |
| $t_{1/2}$ (h) | 2.24 | 1.05 ± 0.225 | 1.89 ± 1.51 | 3.13 ± 1.07 | 4.17 ± 1.27 | 3.29 ± 1.70 | 3.11 ± 0.43 | 2.93 ± 0.476 |
| $C_{max}$ (ng/mL) | 474 | 68.2 ± 31.1 | 113 ± 57.4 | 231 ± 80.6 | 439 ± 76.8 | 354 ± 73.3 | 477.4 ± 150 | 680 ± 134 |
| $T_{max}$ (h) | 0.5 ± 0.25–0.5 | 0.5 ± 0.25–0.5 | 0.25 ± 0.25–1.5 | 0.25 ± 0.25–0.5 | 0.5 ± 0.25–1 | 1 ± 1–2 | 1 ± 1–2 | 2 ± 1–2 |
| F (%) | | 13.9 | 11.9 | 16.0 | 18.4 | | | |

Values are presented as mean ± standard deviation.
bvPLA2, bee venom phospholipase A2; NCBV, new composition bee venom; $AUC_{0-t}$, total area under the plasma concentration-time curve from time zero to time last sampling time; $t_{1/2}$, terminal half-life; $C_{max}$, peak plasma concentration; $T_{max}$, time to reach $C_{max}$, median (ranges); subcutaneous; *F*, bioavailability.

The mean bvPLA2 PK parameters of bvPLA2 in rats following a single SC injection of NCBV at various doses (0.2, 05, 1, and 2 mg/kg) are listed in **Table 1**. After SC injection of NCBV, bvPLA2 was detected in the plasma at the first blood sampling time point (0.05 hours) and reached $C_{max}$ rapidly (0.25–0.5 hours; **Table 1**). The mean plasma concentration of bvPLA2 increased in a dose-dependent manner. Both $C_{max}$ and $AUC_{0-t}$ values displayed a dose-dependent increase.

The mean bvPLA2 PK parameters of bvPLA2 in beagle dogs after single SC injections of 0.3, 0.6, 0.9 mg/kg of NCBV are listed in **Table 1**. Following the SC injection of NCBV, the mean plasma concentration of bvPLA2 increased in a dose-dependent manner. Both the $C_{max}$ and $AUC_{0-t}$ values also increased in a dose-dependent manner.

## Compartmental analysis of bvPLA2 in mice, rats, and beagle dogs

The time course of the plasma bvPLA2 concentrations was best described using a 2-compartment model for mice, rats, and beagle dogs (**Fig. 1**).

A population PK analysis was conducted using the plasma levels of bvPLA2 derived from ICR mice. The final population PK parameters for bvPLA2 are listed in **Table 2**. The mean apparent central volume of distribution ($V_c/F$) was 0.912 L/kg with a relative standard error (RSE) of 5.77%, while the apparent peripheral volume of distribution ($V_p/F$) was 5.89 L/kg with an RSE of 194%. The estimated value for $k_a$ was 9.08 $h^{-1}$ with an RSE of 10.9%. Additionally, the estimated values for Q/*F* (intercompartmental clearance) and CL/*F* were 0.121 L/h/kg (RSE, 106%) and 0.243 L/h/kg (RSE, 56.4%), respectively. The estimated CL/*F* value was similar to that obtained in the non-compartmental analysis (0.353 L/h/kg). Additionally, the IIV in CL/*F* was approximately 13.5% (RSE, 116%).

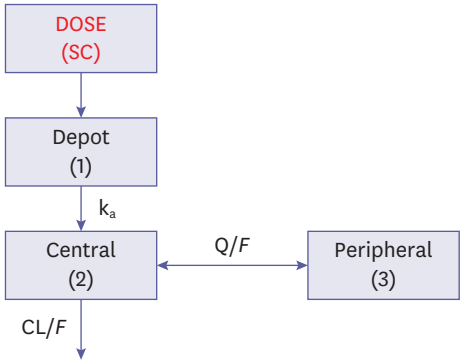


**Figure 1.** Pharmacokinetic model of bvPLA2.
bvPLA2, bee venom phospholipase A2; SC, subcutaneous; $k_a$, absorption rate constant; Q/*F*, intercompartmental clearance; CL/*F*, apparent clearance.


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**Table 2.** Estimates of population pharmacokinetic parameters of bvPLA2 following subcutaneous injection of NCBV into mice, rats and beagle dogs, respectively

| Parameters | Mice (0.5 mg/kg) | | Rats (0.2–0.5 mg/kg) | | Beagle dogs (0.3–0.9 mg/kg) | |
|---|---|---|---|---|---|---|
| | Estimate (% RSE) | BSV (% RSE) | Estimate (% RSE) | BSV (% RSE) | Estimate (% RSE) | BSV (% RSE) |
| CL/*F* (L/h/kg) | 0.243 (56.4) | 13.5 (116) | 1.83 (12.3) | 72.1 (28.2) | 0.150 (19.4) | 27.7 (41.4) |
| $V_c/F$ (L/kg) | 0.912 (5.77) | - | 0.712 (23.5) | - | 0.385 (53.0) | 40.9 (32.3) |
| $V_p/F$ (L/kg) | 5.89 (194) | - | 6.89 (29.3) | - | 1.47 (40.5) | - |
| Q/*F* (L/h/kg) | 0.121 (106) | - | 0.862 (21.7) | 64.5 (41.7) | 0.141 (22.1) | - |
| $k_a$ (1/h) | 9.08 (10.9) | - | 0.803 (15.3) | 35.4 (39.2) | 0.544 (52.0) | - |
| $\sigma_{prop}^3$ (%) | 32.7 (6.42) | - | 32.2 (6.68) | - | 16.6 (6.08) | - |

bvPLA2, bee venom phospholipase A2; NCBV, new composition bee venom; RSE, relative standard error for estimate; BSV, between subject variability; CL/*F*, apparent clearance; $V_c/F$, apparent central volume of distribution; $V_p/F$, apparent peripheral volume of distribution; Q/*F*, intercompartmental clearance; $k_a$, absorption rate constant; $\sigma_{prop}$, proportional error.

A population PK analysis was conducted using the plasma levels of bvPLA2 derived from SD rats. The final population PK parameters for bvPLA2 are listed in **Table 2**. The mean $V_c/F$ was 0.712 L/kg with an RSE of 23.5%, whereas the $V_p/F$ was 6.89 L with an RSE of 29.3%. The estimated value for $k_a$ was 0.803 $h^{-1}$ with an RSE of 15.3%. The estimated Q/*F* and CL/*F* values were 0.862 L/h/kg (RSE, 21.7%) and 1.83 L/h/kg (RSE, 12.3%), respectively. The estimated CL/*F* value was similar to that obtained through the non-compartmental analysis (1.46–2.96 L/h/kg). The IIVs of CL/*F*, Q/*F*, and $k_a$ were 72.1% (RSE, 28.2%), 64.5% (RSE, 41.7%), and 35.4% (39.2%), respectively.

Plasma levels of bvPLA2 derived from beagle dogs were used for population PK analysis. The final population PK parameters for bvPLA2 are listed in **Table 2**. The mean $V_c/F$ was 0.385 L/kg with an RSE of 53.0%, while the $V_p/F$ was 1.47 L/kg with an RSE of 40.5%. The estimated value for $k_a$ was 0.544 $h^{-1}$ with an RSE of 52.0%. The estimated values for Q/*F* and CL/*F* were 0.141 L/h/kg (RSE, 22.1%) and 0.150 L/h/kg (RSE, 19.4%), respectively. The estimated CL/*F* value was similar to that obtained through the non-compartmental analysis (0.164–0.241 L/h/kg). The IIVs of CL/*F* and $V_c/F$ were 27.7% (RSE, 41.4%) and 40.9% (RSE, 32.3%), respectively.

The VPC results for mice, rats, and beagle dogs are shown in **Figs. 2**-**4**, respectively. Most of the observed concentrations fell within the 90% prediction intervals, spanning from the 5$^{th}$ to 95$^{th}$ percentiles, and exhibited a symmetrical distribution around the median. This underscores the model's good predictive performance. However, when a linear elimination process was introduced into the analysis of the rat dataset, the concentration of bvPLA2 in the plasma over time, especially following the 0.2 mg/kg NCBV dose, did not fit well, as depicted in **Fig. 3**.

## Allometric scaling for the prediction of human PK parameters

The results of the allometric regression are listed in **Table 3**. The log-transformed PK parameters exhibited a strong correlation with the log-transformed body weights, with an R-squared value ($R^2$) exceeding 0.781. Using simple allometric scaling approach, the estimated exponents of CL/*F*, $V_c/F$, $V_p/F$, and Q/*F* were 0.847, 0.842, 0.729, and 0.971, respectively. The predicted CL/*F* value was 12.9 L/h in a 70-kg human body and the $R^2$ of the simple allometric scaling approach was 0.781. Subsequently, BrW or MLP were incorporated into allometric scaling, and the CL/*F* for a 70-kg human was better correlated at 3.25 L/h and 0.612 L/h, with the $R^2$ value of 0.973 and 0.904, respectively (**Table 3**). This suggests that incorporating multiple factors and methods may lead to a more refined and accurate estimation of the CL/*F* in a given context.


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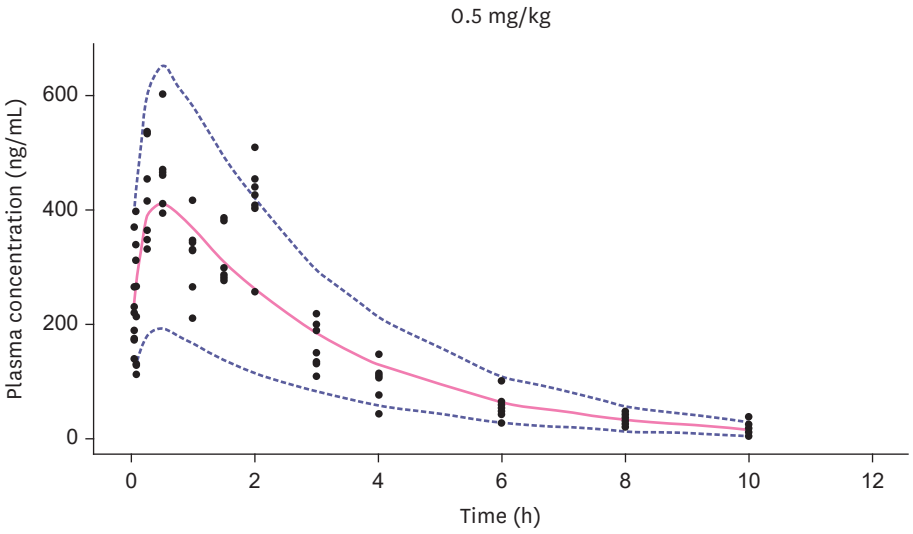


**Figure 2.** VPC of the final model, obtained by SC injecting the NCBV to mice. The solid line corresponds to the 50th percentile of the simulated data, while the lower and upper dashed lines represent the 5th and 95th percentiles of the simulated data, respectively. The circles on the graph represent observational data.
VPC, visual predictive check; SC, subcutaneous; NCBV, new composition bee venom.

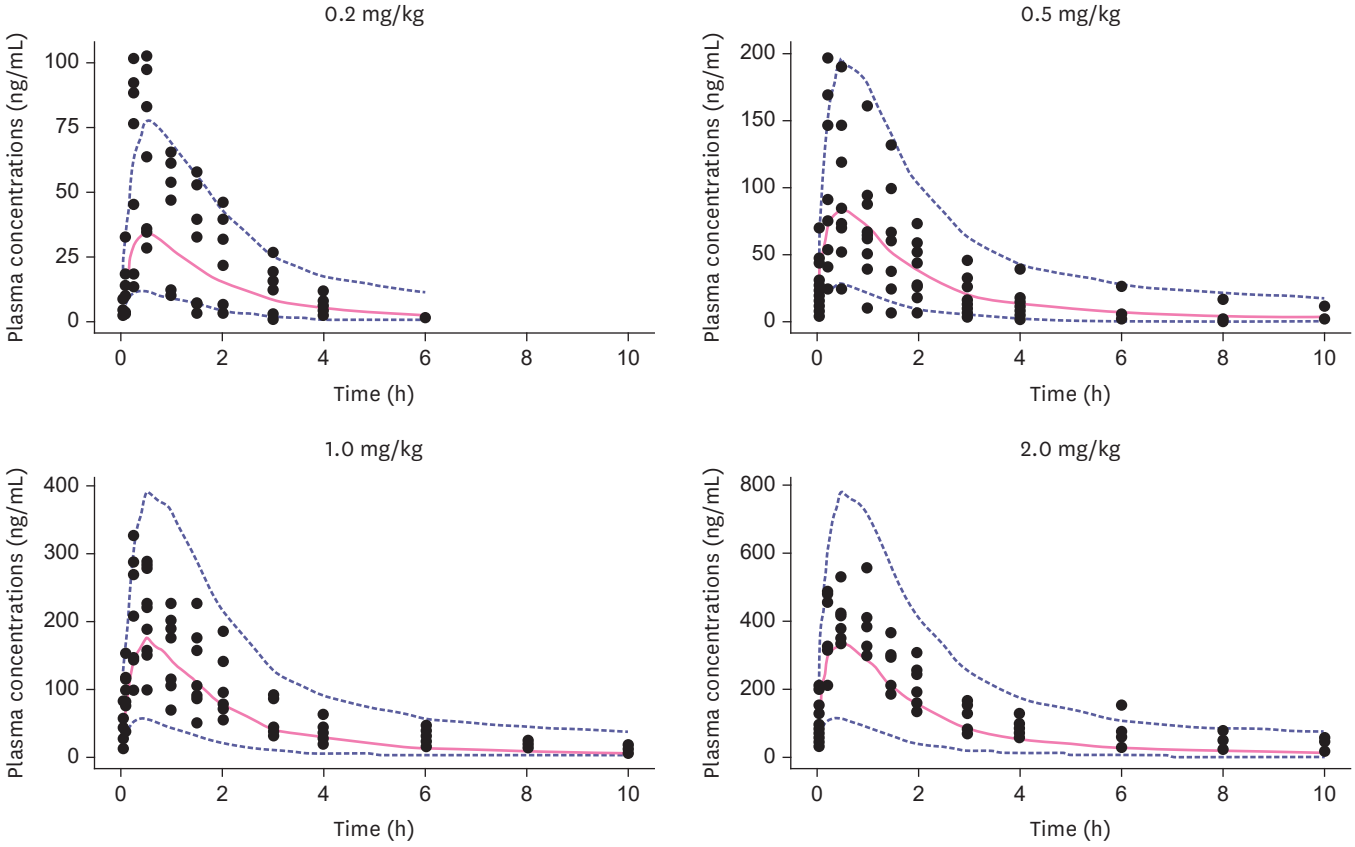


**Figure 3.** VPC of the final model, obtained by SC injecting the NCBV to rats. The solid line corresponds to the 50th percentile of the simulated data, while the lower and upper dashed lines represent the 5th and 95th percentiles of the simulated data, respectively. The circles on the graph represent observational data.
VPC, visual predictive check; SC, subcutaneous; NCBV, new composition bee venom.


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**Table 3.** Allometric scaling of bvPLA2 pharmacokinetic parameters based on data obtained from mice, rats, and beagle dogs

| Parameters | Predicted value in a 70 kg human | $R^2$ |
|---|---|---|
| Simple | | |
| CL/*F* (L/h) | 12.9 | 0.781 |
| $V_c/F$ (L) | 19.5 | 1.00 |
| $V_p/F$ (L) | 67.3 | 0.966 |
| Q/*F* (L/h) | 14.7 | 0.862 |
| BrW | | |
| CL/*F* (L/h) | 3.25 | 0.973 |
| $V_c/F$ (L) | 4.92 | 0.993 |
| $V_p/F$ (L) | 17.0 | 1.00 |
| Q/*F* (L/h) | 3.72 | 0.932 |
| MLP | | |
| CL/*F* (L/h) | 0.612 | 0.904 |
| $V_c/F$ (L) | 10.0 | 0.999 |
| $V_p/F$ (L) | 34.7 | 0.993 |
| Q/*F* (L/h) | 7.59 | 0.940 |

bvPLA2, bee venom phospholipase A2; CL/*F*, apparent clearance; $V_c/F$, apparent central volume of distribution; $V_p/F$, apparent peripheral volume of distribution; Q/*F*, intercompartmental clearance; $k_a$, absorption rate constant; BrW, brain weight; MLP, maximum lifespan potential.

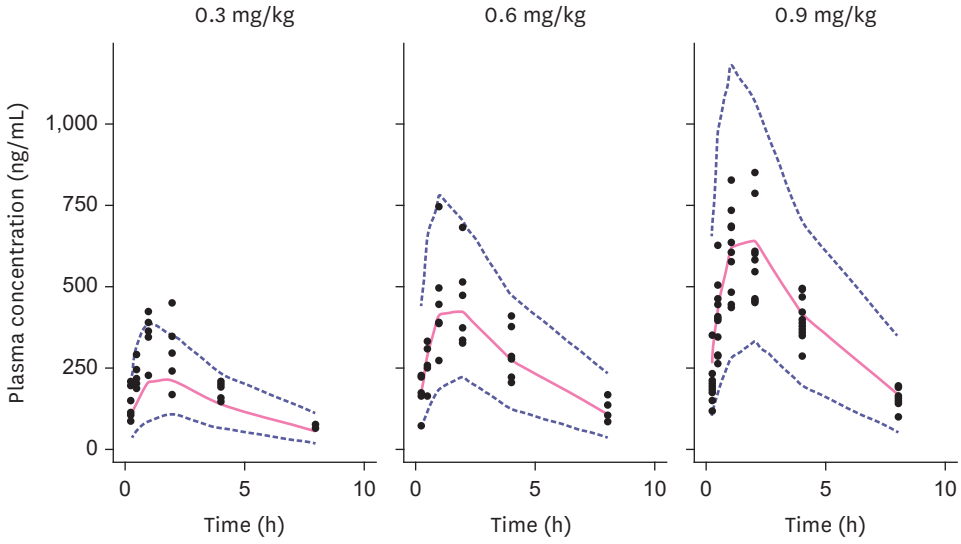


**Figure 4.** VPC of the final model, obtained by SC injecting the NCBV to beagle dogs. The solid line corresponds to the 50th percentile of the simulated data, while the lower and upper dashed lines represent the 5th and 95th percentiles of the simulated data, respectively. The circles on the graph represent observational data.
VPC, visual predictive check; SC, subcutaneous; NCBV, new composition bee venom.

## Human PK simulation and estimation of the FIH dose

Based on the PK parameters, the plasma concentration-time profile of bvPLA2 following a single SC injection of NCBV in humans was simulated using the developed model. Several *in vivo* efficacy studies have been conducted, suggesting that the efficacious dose in MPTP-induced mouse models was greater than 0.1 mg/kg [22]. The calculated AUC using the developed mouse PK model at a dose of 0.1 mg/kg NCBV was 0.397 μg·h/mL. Based on this information, the human equivalent dose (HED) corresponding to the AUC observed at this efficacious dose in mice was explored. The back calculated initial dose for a 70 kg human was 5.5, 1.3, and 3.5 mg, using the simple allometry, BrW, or MLP incorporated model, respectively (**Table 4**). As the BrW method showed the highest coefficient of correlation ($R^2$ value), the predicted FIH dose using the BrW-incorporated human PK model was finally


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**Table 4.** Predicted bvPLA2 pharmacokinetic parameters after single subcutaneous injection of NCBV to a 70-kg human

| Parameters | Simple | BrW | MLP |
|---|---|---|---|
| Dose (mg) | 5.5 | 1.3 | 3.5 |
| $AUC_{0-t}$ (μg·h/mL) | 0.409 | 0.382 | 0.378 |
| $t_{1/2}$ (h) | 7.39 | 7.40 | 3.35 |
| $C_{max}$ (ng/mL) | 78.4 | 73.3 | 64.3 |
| $T_{max}$ (h) | 1 | 1 | 0.5 |
| CL/*F* | 0.01248 | 0.00316 | 0.00917 |

bvPLA2, bee venom phospholipase A2; NCBV, new composition bee venom; BrW, brain weight; MLP, maximum lifespan potential; $AUC_{0-t}$, total area under the plasma concentration-time curve from time zero to time last sampling time; $t_{1/2}$, terminal half-life; $C_{max}$, peak plasma concentration; $T_{max}$, time to reach; $C_{max}$, median (ranges); CL/*F*, apparent clearance.

selected. The predicted dose of 1.3 mg for a 70 kg human fell within the suggested FIH dose range for NCBV, which is from 0.1 to 3 mg, based on the toxicological profile and NOAEL.

The NOAEL approach for estimating the FIH dose was performed by INIST ST (Yongin, Korea) and proceeded as follows. In brief, repeat-dose toxicity studies were conducted in rats and beagle dogs to determine NOAEL. All toxicity studies in rat were conducted by Croen (Suwon, Korea). All toxicity studies in beagle dogs were conducted by ChemOn. The NOAELs for rats (1.88 mg/kg) and dogs (0.6 mg/kg) were converted into HEDs, resulting in 0.30 mg/kg for rats and 0.33 mg/kg for dogs, respectively. The HED was divided by a safety factor of 10 to determine the maximum recommended starting dose (MRSD) [11,12]. For a 70 kg human, the final MRSDs were calculated as 2.1 mg for rats and 2.31 mg for dogs, respectively. Since NCBV is planned to be administered to humans for the first time, the HED calculated from the rat NOAEL, being the lower value, was used. The starting dose was determined by applying a safety factor of 100 to the HED, resulting in 0.21 mg for a 70 kg human. Therefore, the final dose range for the FIH study was proposed to be between 0.1 and 3 mg for a 70 kg human.

Finally, it was concluded that a single SC injection of 1.3 mg NCBV in a 70 kg human would potentially consider both efficacy and safety.

## DISCUSSION

During drug discovery and development, prospective studies for predicting human PKs can provide informed decisions regarding dosing strategies, offering valuable insights for optimizing drug administration in clinical settings. Allometric scaling has proven valuable in determining initial doses in animal studies, particularly in the context of larger animals. These calculations typically use major PK parameters such as CL and $V_d$ obtained from experiments in small animals. The ultimate objective of interspecies PK scaling is to determine the FIH dose.

Prediction of PK parameters was performed using population PK modeling, and a relatively higher RSE for estimate value was observed in the mice model (**Table 2**). This may due to the PK model being built based on the limited PK data from a single dose because of the limited amount of blood that can be collected from mice. Moreover, the parallel cardiac puncture method was used, which led to increased sample variation as the data was obtained from different mice. However, most of the observed concentrations in mice fell within the 90% prediction intervals, ranging from the 5$^{th}$ to 95$^{th}$ percentiles, suggesting that the developed PK model is reliable (**Fig. 2**).


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Furthermore, the developed rat population PK model underestimated the $C_{max}$ after administering 0.2 mg/kg of NCBV. However, the model predicted well with the dose range of 0.5–2 mg and most of the observed concentrations fell within the 90% prediction intervals (**Fig. 3**). When the efficacious dose in mice was converted to rat equivalent dose based on AUC, the predicted dose in rats was 0.7 mg/kg. Therefore, the predictive performance of the PK model is expected to be accurate after injection of the efficacious dose in rat.

NCBV (a fortified content of up to 76.2% bvPLA2) is under development as a new drug for use in patients with Alzheimer's disease. bvPLA2 exhibits a diverse array of pharmacological properties, including antibacterial, anti-cancer, anti-viral, anti-inflammatory, anti-nociceptive, and defensive actions against neurodegenerative diseases [15]. A behavioral experiment investigated whether memory decline, a common symptom of Alzheimer's disease, could be mitigated or reduced following the administration of bvPLA2. A single SC injection of NCBV at a dose of 0.1 mg/kg in MPTP-induced mice resulted in improvement motor activity and suppression of microglial activation, suggesting that bvPLA2 has a potential positive impact on cognitive function [22]. The predicted $AUC_{0-t}$ value was 0.397 μg·h/mL at a dose of 0.1 mg/kg according to the developed PK modeling. The efficacious dose in mice was converted to the human equivalent dose based on AUC, the drug exposure related parameters.

The mathematical analysis in allometric scaling relies on identifying similarities between animals, as well as between animals and humans, in terms of their anatomy, physiology, and biochemistry. Additionally, mathematical analysis for determining interspecies relationships is well established, and CL or $V_d$ can be scaled using a power-law relationship based on the species body weight. Incorporating additional factors such as BrW or MLP into the equations can further enhance interspecies correlations [23-25]. In this study, allometric scaling was performed arbitrarily using SC data owing to the lack of IV data from mice and beagle dogs. IV PK data was only available for rats and allometric scaling was conducted using CL/*F* and V/*F*, assuming that *F* is consistent across all species. However, given that the observed *F* in rats ranges from 11.9% to 18.4%, which is below 20%, it is expected that a similar value is reflected in the scaled CL/*F* and V/*F* values. To more accurately account for human *F*, IV data from not only rats but also mice and dogs would be necessary.

**Table 3** shows the predicted CL/*F*, $V_c/F$, $V_p/F$, and Q/*F* values in a 70-kg human derived via simple, BrW, or MLP incorporated allometric scaling from mice, rats, and beagle dogs. Log-transformed body weight and PK parameters demonstrated a good correlation when using the BrW and MLP incorporated methods. (e.g. CL/*F*; $R^2$ = 0.973 and 0.904, respectively). On the other hand, the log-transformed CL/F parameter derived using the simple method did not showed good correlation ($R^2$ = 0.781), indicating a potential discrepancy or divergence in the correlation of this specific parameter in the simple method [26].

The predicted initial doses for a human with 70 kg body weight were 5.5, 1.3, and 3.5 mg when using the simple, BrW, and MLP incorporated method, respectively. The simulated plasma concentration-time profiles of bvPLA2 in humans after injection of 5.5, 1.3, and 3.5 mg NCBV were in line with the plasma concentration-time profiles in mice after injection of 0.1 mg/kg, indicating that the HED at the efficacious dose in mice was reasonably predicted (**Fig. 5**). Moreover, suggested FIH dose range for NCBV was from 0.1 to 3 mg, based on the toxicological profile and the NOAEL and participant's safety should always be the top priority in clinical trials [12]. Our predicted FIH dose was within the NOAEL based FIH dose range, indicating that the proposed dose is expected to be both efficacious and safe.


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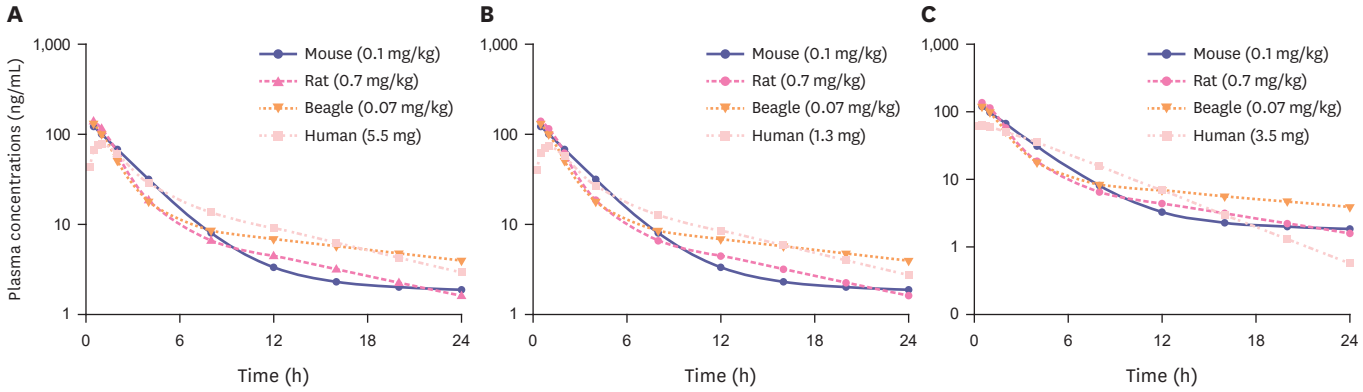


**Figure 5.** The estimated plasma concentrations-time profiles of bvPLA2 in humans following a single subcutaneous injection of NCBV. The data were obtained using 3 different approaches: simple (A), BrW (B), and MLP (C).
bvPLA2, bee venom phospholipase A2; NCBV, new composition bee venom; BrW, brain weight; MLP, maximum lifespan potential.

In conclusion, the suggested SC dose for the FIH study is 1.3 mg of NCBV for a 70-kg human. The predicted FIH dose was defined using BrW incorporated allometric approach, which showed a good correlation ($R^2 = 0.973$) with different species. Our proposed efficacious dose lies within the phase I dose ranges established by safety criteria. Due to the absence of IV data from mice and beagle dogs, PK parameters were explained by dividing *F*. Refinement of the PK model by incorporating *F* might lead to a better allometric relationship and a more accurate prediction of FIH dose.

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