Calculation of a First-In-Man Dose of 7-O-Succinyl Macrolactin A Based on Allometric Scaling of Data from Mice, Rats, and Dogs

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Abstract
7-O-Succinyl macrolactin A (SMA) exerts several pharmacological effects including anti-bacterial, anti-inflammation, and anti-cancer activities. Recently, SMA has been extensively evaluated as an anti-cancer drug. Thus, the objectives of the present study were to characterise the pharmacokinetics of SMA via both non-compartmental and compartmental analysis in mice, rats, and dogs, and to derive an appropriate first-in-man dose based on allometric scaling of the animal data. The time courses of plasma SMA concentrations after intravenous administration to rats and dogs were analysed retrospectively, as were data collected after intraperitoneal SMA injection in mice. Pharmacokinetic parameters were estimated via both noncompartmental and compartmental analysis, and were correlated with body weight and/or the potential maximum life-span. The clearance and distribution volume of SMA in humans were predicted, and a first-in-man dose proposed. A two-compartment model best described the time courses of SMA plasma concentrations after a saturation elimination process was applied to fit the dataset obtained from rats. Incorporation of the maximum potential life-span during allometric scaling was required to improve the estimation of human clearance. The SMA clearance and the distribution volume in the steady state, in a 70-kg adult male, were estimated to be 30.6 L/h and 19.5 L, respectively. To meet the area under the curve (AUC) required for anti-tumour activity, a dose of 100 mg (~1.5 mg/kg) was finally proposed as the first dose for a 70-kg human. Although toxicological profiles derived from non-clinical studies must be considered before any final decision is made, our work will facilitate clinical studies on SMA.

Key Words: 7-O-Succinyl macrolactin A, Pharmacokinetics, First-In-Man dose, Allometric scaling, Dienetichron plot

INTRODUCTION

7-O-Succinyl macrolactin A (4-[(3Z,5E,8S,9E,11Z,14S,16R,17E,19E,24R)-14,16-dihydroxy-24-methyl-2-oxo-1-oxacyclotetraacosa-3,5,9,11,17,19-hexaen-8-yl]oxy)-4-oxobutanoic acid, SMA, Fig. 1) is a polyene macrolide containing a 24-member lactone ring, and is usually isolated from marine bacteria including Streptomyces, Actinomadura, and Bacillus. Recently, SMA was isolated at high yield from a fermentation broth of Bacillus polyfermenticus KJS-2 (Kim et al., 2009).

SMA exerts anti-bacterial effects against vancomycin-resistant enterococci (minimum inhibitory concentration [MIC], 2 mg/L) and methicillin-resistant Staphylococcus aureus (MIC, <0.25 mg/L). SMA was much more potent than teicoplanin,
which is used to treat vancomycin-resistant enterococcal infections (Kim et al., 2011). SMA also exerts a protective effect on intestinal inflammation (Park et al., 2014). Recently, SMA was shown to exert significant anti-angiogenic effects on human umbilical vein endothelial cells, which may be attributable to inhibition of the vascular endothelial growth factor (VEGF)-induced angiogenic process, proliferation, tube formation, and invasion. Intraperitoneal injection of SMA into tumour-bearing mice (the CT26 mouse colon cancer allograft model) afforded dose-dependent anti-tumour activity, and the survival rate was significantly extended (Regmi et al., 2015). SMA has been modified via formation of the Tris salt to improve water solubility, and the safety of this formulation is being evaluated in non-clinical studies.

A major issue in drug development is determination of an appropriate safe commencement dose for humans. Allometric scaling is based on the facts that energy requirements and the rates of physiological processes are closely associated with body size. Such scaling has been useful to predict pharmacokinetic parameters, including the clearances, distribution volumes, and half-lives, of drug candidates in humans, using data derived from various animals (Mordenti, 1986; Obach et al., 1997; Lin et al., 1999; Khor et al., 2000; Kelley et al., 2001). Such an approach is particularly useful when putative anticancer agents have narrow safety margins.

The pharmacokinetic profiles of SMA in mice and rats were previously examined by other researchers (Kim et al., 2013; Jung et al., 2014). Our group also reported on the pharmacokinetic behaviour of SMA in rats and dogs, using a sensitive and validated method to examine drug stability in plasma (Kim et al., 2014; Noh et al., 2014). In the present work, we conducted a pharmacokinetic analysis of SMA in mice to explore differences in data from various laboratories, which may be attributable to the analytical methodology chosen, or differences in animal care. Pharmacokinetic datasets from mice, rats, and dogs were analysed retrospectively.

The principal aims of the current study were to explore the relationships between SMA pharmacokinetic parameters, and physiological factors, in mice, rats, and dogs; to model the time courses of plasma SMA concentrations via computational modelling; and, finally, to predict SMA pharmacokinetic parameters in humans and define an appropriate first-in-man dose for clinical trials.

**MATERIALS AND METHODS**

**Materials**

The Tris salt of SMA (purity, 98.4%) was supplied by Daewoo Pharm. Co. Ltd (Busan, Korea). Methaqualone (the internal standard, IS) and sodium fluoride (NaF) were purchased from Sigma (Seoul, Korea). Acetonitrile and methanol were obtained from Burdick & Jackson (Muskegon, MI, USA). All other chemicals and solvents were of the highest available analytical grade.

**Animals**

Pharmacokinetic studies were conducted in 25 male ICR mice (28-34 g). The animal room was maintained at a temperature of 23 ± 3°C, a relative humidity of 50 ± 10% with 10-20 air changes/h, and a light intensity of 150-300 Lux with a 12-h light/dark cycle. The study was approved by the Yeungnam University Animal Care and Use Committee (Gyeongsan, Korea). All animals used in this study were cared for in accordance with the principles outlined in the National Institutes of Health Guide for the Care and Use of Laboratory Animals.

**Pharmacokinetic experiments**

SMA salt dissolved in saline was administered to male ICR mice via single intraperitoneal injections (100 mg/kg). As described earlier, single intravenous injections were also given to 15 male Sprague-Dawley rats (10, 30, and 90 mg/kg) and three male beagle dogs (25 mg/kg). Blood samples were obtained from the retro-orbital plexus of mice (100 µL; 1-2 samples from each mouse) and from the jugular vein of rats (200 µL) and dogs (1 mL). To ensure SMA stability (Noh et al., 2014), samples were collected in heparinised tubes containing NaF (final concentration 1 mg/mL). Samples were collected at 2, 5, 10, 15, 30, 45, 60, 90 and 120 min following SMA administration in mice; at 2, 5, 10, 20, 30, 45, 60, 75, 90 and 120 min in rats; and at 2, 5, 15, 30, 45, 60, 90, 120, 180, 240 and 360 min in dogs. After centrifugation at 13,200 rpm for 10 min, plasma was separated and stored at -20°C.

**Analytical method**

Plasma concentrations of SMA were determined using a validated analytical method (Noh et al., 2014). Briefly, SMA was quantified with the aid of an API 4000 LC-MS/MS system (AB SCIEX, Framingham, MA, USA) equipped with an electrospray ionisation interface, and operating in the positive-ion mode. The compounds were separated on a reverse-phase column (ZIC® HILIC, 50×2.1 mm internal diameter, 5-µm particle diameter; Merck, Darmstadt, Germany) featuring a mobile phase (10 mM formic acid in water, methanol, and acetonitrile; 15:15:70 v/v/v). The column was heated to 40°C, and the flow rate was held at 0.3 ml/min using an HP 1260 series pump (Agilent, Wilmington, DE, USA). SMA was identified principally as the sodium adduct ion [M+Na] at m/z 525.4, and the IS yielded primarily protonated molecules [M+H]+ at m/z 251.1. The product ions were scanned in Q3 after collision with nitrogen in Q2 at m/z 406.7 and 132.1 for SMA and the IS, respectively. Quantification was achieved by selective monitoring of SMA sodium adducts or protonated precursor ions, and associated product ions, with derivation of the ratios of the areas under the peaks for each solution. Analytical data were processed with the aid of Analyst software (version 1.5.2, Applied Biosystems, Foster City, CA, USA).

The IS (150 µL; 1 ng/mL in methanol) was added to 30 µL of plasma, vortexed for 30 s, and centrifuged (13,200 rpm, 10 min). Next, a 3-µL aliquot of the supernatant was injected into the column. The lower limit of quantitation (LLOQ) was 20 ng/mL and plasma samples that were expected to contain high levels of SMA were diluted 20- or 100-fold with blank plasma (Noh et al., 2015, 2016).

**Model-independent analysis**

The SMA pharmacokinetic parameters were obtained via analysis of the time course of plasma concentrations in mice following single intraperitoneal injections of Tris SMA, and in rats and dogs following single intravenous injections. Model-independent pharmacokinetic parameters were calculated via non-compartmental analysis using WinNonlin® (version 2.1, Scientific Consulting, Palo Alto, CA, USA); the parameters derived included the maximum concentration (Cmax), the time
to maximum concentration ($T_{\text{max}}$), the area under the plasma concentration-time curve from the time of dosing extrapolated to infinity (the AUC$\infty$), the total body clearance (CL), the distribution volume ($V_d$), and the terminal half-life ($t_1/2$) (Chiu, 1978). The pharmacokinetic parameters obtained from each rat and dog were expressed as means ± standard deviations. The naïve-pooled method was used to describe the pharmacokinetics of SMA in mice (Kim and Kang, 2013).

Model-dependent analysis

Population pharmacokinetic modelling was performed using the subroutine ADVAN13 and the first-order conditional estimation with interaction (FOCE-I) methods of NONMEM® (version 7.2; ICON Development Solutions, MD, USA) running Pirana® (version 2.8.2, ActiveState, Vancouver, Canada) (Bauer, 2011; Keizer et al., 2013). The SMA plasma concentration-time curves following single intraperitoneal injections of Tris SMA (100 mg/kg) were described using a two-compartment model featuring a first-order absorption and elimination. Based on a previous report by Jung et al. (2014), the absolute bioavailability (F) for the concentration-time curves was fixed at 0.2. The time courses of plasma SMA concentrations after intravenous administration of Tris SMA to rats and dogs were characterised by a two-compartment model with non-linear elimination described by the Michaelis-Menten equation, and first-order elimination, respectively. Observations below the LLOQ were excluded from the population pharmacokinetic modelling analyses.

The inter-individual variabilities of pharmacokinetic parameters were derived using the following exponential error model:

$$\log Y = \log(a) + b \times \log(W)$$

where $Y$ is the pharmacokinetic parameter of interest, $W$ the body weight, and $a$ and $b$ the coefficient and exponent, respectively, of the allometric equation (Mahmood and Balian, 1996b). The allometric relationship may be linearised when plotted using log-log coordinates:

$$\log Y = \log(a) + b \times \log(W)$$

where $\log(a)$ is the y-intercept and $b$ the slope of the relationship.

The total clearance, and the central and steady-state distribution volumes, estimated via compartmental analysis in mice, rats, and dogs, were used to predict these parameters in a human. Importantly, we used the total SMA clearance obtained via non-compartmental analysis in rats that received 30 mg/kg Tris SMA intravenously to predict the human clearance, because SMA elimination by rats was nonlinear as the dose rose. The mean value of each pharmacokinetic parameter and the mean species weight were plotted using log-log coordinates. A linear relationship was fitted to the log-transformed data to estimate the parameters $a$ and $b$.

If necessary, the maximum potential life-span (MLP) and/or brain weight were incorporated into the equations, depending on the value of the clearance exponent derived via simple allometry.

Finally, the total clearance estimated by allometric scaling was used to predict the first-in-man dose of SMA. Clearance was multiplied by the AUC (~200 mg·min/L) to ensure sufficient anti-tumor activity (Kang et al., 2012).

Dedrick plots

Since intravenous administration of 10 and 30 mg/kg of SMA in rats and 25 mg/kg in dogs yielded linear pharmacokinetics, the SMA plasma concentration-time profiles were predicted using three Dedrick plots (Dedrick et al., 1970), including kallikreinchrons (elementary Dedrick plots), apolysichrons (complex Dedrick plots) (Boxenbaum and Ronfeld, 1983), and dienetichrons (Boxenbaum, 1983), following a single intra-
venous injection of Tris salt in humans (1.5 mg/kg). The plot
equations were as follows:

Kallynochrons (elementary Dedrick plots):

\[
\text{Time}_H = \text{Time}_A \times \left( \frac{W_H}{W_A} \right)^{1-b}
\]

\[
\text{Conc}_H = \text{Conc}_A \times \left( \frac{W_H}{W_A} \right)^c
\]

Apolysichrons (complex Dedrick plots):

\[
\text{Time}_H = \text{Time}_A \times \left( \frac{W_H}{W_A} \right)^{c-b}
\]

\[
\text{Conc}_H = \text{Conc}_A \times \left( \frac{W_H}{W_A} \right)^c \times \left( \frac{\text{Dose}_H}{\text{Dose}_A} \right) \times \left( \frac{W_A}{W_H} \right)^b
\]

Dienetichrons:

\[
\text{Time}_H = \text{Time}_A \times \left( \frac{\text{MLP}_H}{\text{MLP}_A} \right) \times \left( \frac{W_H}{W_A} \right)^{c-d}
\]

\[
\text{Conc}_H = \text{Conc}_A \times \left( \frac{\text{Dose}_H}{\text{Dose}_A} \right) \times \left( \frac{W_A}{W_H} \right)^c
\]

where \(W\) is the body weight in kg. The subscripts \(H\) and \(A\) indicate human and animal, respectively. The superscripts \(b, c,\) and \(d\) are the exponents derived upon simple allometry of \(CL\) and \(V_{ss}\), and allometry of \(CL\timesMLP\), across rats and dogs, respectively. MLP is the maximum potential life-span in h, and dose is the intravenous dose of SMA in milligrams. The human concentration-time curves predicted by the Dedrick plots were used to calculate human pharmacokinetic parameters including \(CL, V_{ss},\) and \(t_{1/2}\) employing a two-compartment model weighted with the squared reciprocal of the predicted concentration \((1/y^2)\), using WinNonlin® (version 2.1, Scientific Consulting). The goodness of curve fitting for all Dedrick plots was evaluated using the Akaike Information Criterion (AIC) and visual examination.

RESULTS

Non-compartmental analysis of SMA in mice, rats, and dogs

The mean SMA plasma concentration-time profiles in mice after a single intraperitoneal injection of 100 mg/kg of the drug salt are shown in Fig. 2A. The SMA pharmacokinetic parameters are listed in Table 1. SMA was rapidly absorbed, and the plasma concentration thereof decayed bi-exponentially. The peak concentration \((C_{\text{max}})\) was 24.8 mg/L at 5 min. The \(t_{1/2}\) was approximately 18.2 min, and the \(V_{ss}\) and total \(CL\) were 5.0 L/kg and 0.2 L/min/kg, respectively. The estimated \(AUC_{\infty}\) was 423.2 mg·min/L.

The mean plasma concentration-time profiles of SMA in rats after single intravenous injections of 10, 30, and 90 mg/kg of the drug salt are shown in Fig. 2B. The pharmacokinetic parameters of SMA are listed in Table 1. The plasma concentrations of SMA exhibited a bi-exponential decay profile, as in mice, and the mean half-lives and distribution volumes ranged from 14-25 min and 0.2-0.33 L/kg, respectively. The systemic clearance of SMA fell with increasing dose (0.049 ± 0.018, 0.034 ± 0.007, and 0.018 ± 0.002 L/min/kg, respectively), and the \(AUC_{\infty}\) increased exponentially (185.7 ± 71.1, 734.5 ± 177.6, and 3,992.3 ± 359.5 mg·min/L, respectively).

The mean plasma concentration-time profile of SMA in dogs after single intravenous injections of 25 mg/kg of the drug salt is shown in Fig. 2C. The pharmacokinetic parameters of SMA
are listed in Table 1. As was also true of mice and rats, the plasma concentration of SMA decayed bi-exponentially, but the terminal half-life (55.0 ± 19.2 min) thereof was much longer in dogs than in the other animals. Vd and CL were 0.28 ± 0.03 L/kg and 0.026 ± 0.002 L/min/kg, respectively. The AUC∞ was 777.1 ± 65.5 mg·min/L.

Compartmental analysis of SMA in mice, rats, and dogs

The time courses of plasma SMA concentrations were best described by a two-compartment model featuring linear elimination for mice (Fig. 3A) and dogs (Fig. 3B), and saturable elimination for rats (Fig. 3C).

Population pharmacokinetic analysis evaluated 45 SMA

Table 1. Model-independent SMA pharmacokinetic parameters following intraperitoneal (i.p.) or intravenous (i.v.) injection of the SMA Tris salt into mice, rats, and dogs

| Parameter          | Mice 100 mg/kg, i.p. | Rats 10 mg/kg, i.v. | Rats 30 mg/kg, i.v. | Rats 90 mg/kg, i.v. | Dogs 25 mg/kg, i.v. |
|--------------------|----------------------|---------------------|--------------------|--------------------|---------------------|
| Cmax (mg/L)        | 24.8                 | -                   | -                  | -                  | -                   |
| Tmax (min)         | 5.0                  | -                   | -                  | -                  | -                   |
| t1/2 (min)         | 18.2                 | 17.0 ± 10.6         | 24.5 ± 10.2        | 14.1 ± 2.0         | 55.0 ± 19.2         |
| Vd/F (L/kg)        | 5.0                  | 0.33 ± 0.13         | 0.27 ± 0.06        | 0.20 ± 0.01        | 0.28 ± 0.03         |
| CL/F (L/min/kg)    | 0.2                  | 0.049 ± 0.018       | 0.034 ± 0.007      | 0.018 ± 0.002      | 0.026 ± 0.002       |
| AUC∞ (mg·min/L)    | 423.2                | 185.7 ± 71.1        | 734.5 ± 177.6      | 3992.3 ± 359.5     | 777.1 ± 65.5        |

Data are expressed as mean ± SDs (n=5), except for the naïve-pooled mean values obtained from three different mice.

Table 2. Estimates of population pharmacokinetic SMA parameters following intraperitoneal injection of the SMA Tris salt (100 mg/kg) into mice

| Parameter          | Estimate (%RSE\(^a\)) | Bootstrap median (95% CI\(^b\)) |
|--------------------|------------------------|---------------------------------|
| Structural model   |                        |                                 |
| k1 (h\(^{-1}\))    | 15.3 (7)               | 15.4 (9.2–19.9)                 |
| CL (L/h/kg)        | 2.39 (4)               | 2.39 (2.21–2.60)                |
| V1 (L/kg)          | 0.296 (9)              | 0.289 (0.179–0.408)             |
| V2 (L/kg)          | 0.384 (6)              | 0.380 (0.329–0.531)             |
| Q (L/h/kg)         | 1.57 (14)              | 1.56 (1.15–2.23)                |
| Inter-individual variability |          |                                 |
| \(\omega_{CL}\)   | 13.9% (21)             | 13.7% (5.0–19.1)                |
| Residual error     |                        |                                 |
| \(\sigma_{add}\) for SMA | 0.0111 (16)          | 0.0144 (0.0019–0.1457)          |
| \(\sigma_{prop}\) for SMA | 0.216 (22)             | 0.189 (0.074–0.272)             |

\(^a\)%RSE, relative standard error for estimate.

\(^b\)CI, confidence interval calculated from 1000 bootstrap resamplings.

Table 3. Estimates of population pharmacokinetic SMA parameters following intravenous injection of the SMA Tris salt (10, 30, and 90 mg/kg) into rats

| Parameter          | Estimate (%RSE\(^a\)) | Bootstrap median (95% CI\(^b\)) |
|--------------------|------------------------|---------------------------------|
| Structural model   |                        |                                 |
| Vmax (mg/h/kg)     | 260 (20)               | 266 (190–537)                   |
| Km (mg/L)          | 78.1 (28)              | 80.1 (51.1–209.6)               |
| V1 (L/kg)          | 0.173 (14)             | 0.169 (0.130–0.216)             |
| V2 (L/kg)          | 0.201 (9)              | 0.198 (0.152–0.232)             |
| Q (L/h/kg)         | 1.31 (20)              | 1.31 (0.85–1.80)                |
| Inter-individual variability |          |                                 |
| \(\omega_{Vmax}\) | 14.6% (33)             | 13.5% (6.8–24.1)                |
| Residual error     |                        |                                 |
| \(\sigma_{add}\) for SMA | 0.0466 (26)             | 0.0463 (0.0233–0.0796)          |
| \(\sigma_{prop}\) for SMA | 0.192 (15)             | 0.185 (0.130–0.235)             |

\(^a\)%RSE, relative standard error for estimate.

\(^b\)CI, confidence interval calculated from 1000 bootstrap resamplings.
plasma levels derived from 25 ICR mice. The final population pharmacokinetic parameters of SMA are shown in Table 2. The mean central ($V_c$) and peripheral ($V_p$) distribution volumes were 0.296 L/kg (RSE, 9%) and 0.384 L (RSE, 6%), respectively. The estimated $k_a$ was 15.3 h$^{-1}$ (RSE, 7%). $Q$ and $CL$ were estimated to be 1.57 L/h/kg (RSE, 14%) and 2.39 L/h/kg (RSE, 4%), respectively. The estimated CL value was similar to that obtained upon non-compartmental analysis that considered the bioavailability of SMA (20%). The CL inter-individual variability was $\sim$14%.

Population pharmacokinetic analysis evaluated 141 SMA plasma levels derived from 15 rats. The final population pharmacokinetic parameters of SMA are shown in Table 3. The mean central ($V_c$) and peripheral ($V_p$) distribution volumes were 0.173 L/kg (RSE, 14%) and 0.201 L/kg (RSE, 9%), respectively. The $V_{ss}$ (sum of $V_c$ and $V_p$) values were similar to those obtained via non-compartmental analysis. The $Q$, $V_{max}$, and $K_m$ estimates were 1.31 L/h/kg (RSE, 20%), 260 mg/h/kg (RSE, 20%), and 78.1 mg/L (RSE, 28%), respectively. The inter-individual variability of $V_{max}$ was $\sim$15%.

Twenty-seven SMA plasma levels from three beagle dogs were subjected to population pharmacokinetic analysis. The final population pharmacokinetic parameters for SMA are shown in Table 4. The mean central ($V_c$) and peripheral ($V_p$) distribution volumes were 0.31 L/kg (RSE, 6%) and 0.108 L (RSE, 2%), respectively. The $Q$ and $CL$ estimates were 0.068 L/h/kg (3%) and 1.4 L/h/kg (6%), respectively. The estimated $CL$ and $V_{ss}$ (the sum of $V_c$ and $V_p$) were similar to those obtained via non-compartmental analysis. Inter-individual variabilities in parameters were neglected because the sample

| Parameter          | Estimate (%)RSE\(^a\) | Bootstrap median (95% CI\(^b\)) |
|--------------------|------------------------|---------------------------------|
| Structural model   |                        |                                 |
| $CL$ (L/h/kg)      | 1.7 (6)                | 1.7 (1.5~1.9)                   |
| $V_c$ (L/kg)       | 0.31 (6)               | 0.31 (0.27~0.33)                |
| $Q$ (L/h/kg)       | 0.108 (2)              | 0.106 (0.079~0.111)             |
| Residual error     | $\sigma_{prop}$ for SMA | 0.288 (7)                      |
|                    |                        | 0.275 (0.191~0.309)             |

\(^a\)%RSE, relative standard error for estimate.
\(^b\)CI, confidence interval calculated from 1000 bootstrap resamplings.

Fig. 4. Visual predictive check of the final model derived by injecting the SMA Tris salt into mice (A), dogs (B), and rats (C). Panel (D) shows a model fitted by linear elimination rather than the nonlinear process used to derive Fig. 4C. The solid, and upper and lower dashed, lines indicate the 50th, 95th, and 5th percentiles of the simulated data, respectively. The circles represent observational data.
The observed bootstrap medians of SMA pharmacokinetic parameters were generally consistent with the mean population estimates. Basic goodness-of-fit plots for the final pharmacokinetic model showed that individually predicted SMA concentrations corresponded well to observations made in the absence of systemic bias, except for high drug concentrations in dogs. The conditional weighted predictions for the final population pharmacokinetic model were generally distributed around zero, and were relatively symmetrical (data not shown). The VPC results following single intraperitoneal injections into mice and single intravenous injections into dogs and rats are shown in Fig. 4A to 4C, respectively. Most observed concentrations were within the 90% prediction intervals (the 5th-95th percentiles), and were symmetrically distributed around the median, indicating that the model afforded good predictive performance. When a linear elimination process was incorporated into evaluation of the rat dataset, the time course of plasma SMA concentrations (especially after the 90 mg/kg dose) fitted poorly (Fig. 4D).

**Allometric scaling**

The results of allometric regression are listed in Table 5 and depicted in Fig. 5 and 6. The log-transformed pharmacokinetic parameters correlated well with the log-transformed body weights ($r^2$>0.994); and the estimated exponents for $CL$, $V_{ss}$, and $V_{c}$ were 0.9406, 0.9236, and 1.0208, respectively. Using simple allometric scaling, the predicted values were 104.9 L/h

**Table 5. Allometric scaling of SMA pharmacokinetics based on data derived from mice, rats, and dogs**

| Parameter | Allometric equation | $r^2$ | Predicted value in a 70 kg human (95% CI) | Method |
|-----------|---------------------|-------|----------------------------------------|--------|
| $CL$ (L/h) | $1.93\times(WT)^{0.9406}$ | 1.0000 | 104.9 (98.9~111.4) | Simple allometry |
| $CL$ (L/h) | $10^{5.1476\times(WT)^{1.2205}/MLP}$ | 0.9990 | 30.6 (14.6~64.1) | $CL\times MLP$ |
| $V_{ss}$ (L) | $0.45\times(WT)^{0.9236}$ | 0.9962 | 22.5 (7.4~69.0) | Simple allometry |
| $V_{c}$ (L) | $0.26\times(WT)^{1.0208}$ | 0.9941 | 19.5 (4.2~91.6) | Simple allometry |

*CI, confidence interval; WT and MLP represent body weight and maximum life-span potential, respectively.

(size was small.

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The estimated exponent for CL was 0.9406, indicating that the CL estimate was greater than the actual value (Mahmood and Balian, 1996b). Therefore, the rule of exponents function was used to improve the prediction performance (Mahmood and Balian, 1996b). Thus, the human CL was calculated by dividing by the human MLP (8.18×10^5 h) (Mahmood, 2007):

\[
CL_{\text{human}} = \frac{a \times W^b}{8.18 \times 10^5}
\]

The MLP was obtained in years by the following equation:

\[
\text{MLP(years)} = 185.4 \times (BW)^{0.636} \times W^{-0.225}
\]

After considering the MLP (Fig. 5B), the human CL was more accurately calculated as 30.6 L/h (0.51 L/min) in a 70-kg human. Thus, the proposed first-in-man dose for a 70-kg human would be 100 mg (~1.5 mg/kg), which was calculated by multiplying the AUC (200 mg·min/L) by the clearance (0.51 L/min).

Table 6. Predicted SMA pharmacokinetic parameters following intravenous administration of 1.5 mg/kg of the SMA Tris salt to a human (70 kg), derived using Dedrick plots

| Parameter | Kallynochrons | Apolysichrons | Dienetichrons |
|-----------|---------------|---------------|---------------|
| CL (L/h)  | 145.7 (104.1–187.4)* | 148.0 (103.3–192.7) | 38.7 (29.0–48.4) |
| Vss (L)   | 27.3 (14.0–40.5) | 19.2 (9.5–28.9) | 20.2 (11.7–28.8) |
| t1/2 (h)  | 0.31 (0.24–0.38) | 0.22 (0.17–0.27) | 0.88 (0.71–1.04) |
| AUC (mg·h/L) | 0.6 (0.4–0.7) | 0.6 (0.4–0.7) | 2.2 (1.6–2.7) |
| AIC       | -280.7 | -277.4 | -288.3 |

*Estimate (90% confidence interval), AIC, Akaike Information Criteria.
Dedrick plots
The results of the Dedrick plots are listed in Table 6, and the predicted plasma concentration-time profile of SMA in humans following a single intravenous administration of 1.5 mg/kg of the drug salt is shown in Fig. 7; data from three Dedrick plots are displayed. The predicted SMA pharmacokinetic parameters based on both elementary and complex Dedrick plots were 145.7 and 148.0 L/h for CL, 27.3 and 19.2 L for Vss, and 0.31 and 0.22 h for t1/2, in a 70-kg human. All estimated values were similar regardless of whether elementary or complex Dedrick plots were prepared. The dienetichron plot predicted that CL, Vss, and t1/2 in a 70-kg human would be 38.7 L/h, 20.2 L, and 0.88 h, respectively.

DISCUSSION
Allometric scaling has been useful to define first-in-animal doses, especially in large animals. Such calculations use the values of major pharmacokinetic parameters (clearance and distribution volume) obtained in small experimental animals. However, the ultimate goal of interspecies pharmacokinetic scaling is to determine first-in-man doses. The mathematical analysis is based on similarities between animals, and animals and humans, in terms of anatomy, physiology, and biochemistry. Such interspecies relationships are well-established; drug disposition and/or clearance can be simply scaled, using a power-law relationship, by body weight, and the MLP and/or brain weight can be incorporated into the equations to improve interspecies correlations (Mordenti, 1986).

SMA is under investigation as an anti-cancer drug; non-clinical safety studies are currently in progress. In the present study, the time courses of plasma SMA concentrations in mice, rats, and dogs following intraperitoneal or intravenous administration of the Tris salt were analysed using both non-compartmental and compartmental approaches. Human pharmacokinetic SMA parameters were predicted via allometric scaling, and a first-in-man dose was finally derived.

As SMA exhibited promising anti-tumour activity upon repeated intraperitoneal administration (50 mg/kg) to tumour-bearing mice (Kang et al., 2012), and as the intraperitoneal bioavailability of SMA in mice is ~0.2 (Jung et al., 2014), the SMA dose for rats was increased from 10 mg/kg to 90 mg/kg, and a half-dose (25 mg/kg) was given to dogs, to obtain pharmacokinetic parameters. As shown in Table 1, the clearance in rats decreased, and systemic exposure to SMA (as reflected by the AUC) exponentially increased, with increasing doses, indicating saturation of drug metabolism and/or excretion. Therefore, a Michaelis-Menten-type elimination process was used in compartmental modelling; the model successfully described the time courses of plasma SMA concentrations at all tested doses. Vss and Kd were estimated to be 260 mg/h/kg and 78 mg/L, respectively, and the intrinsic clearance (Vss/Kd) was 3.3 L/h/kg (0.055 L/min/kg), comparable to the systemic clearance (0.04 L/min/kg). This explains why the drug bioavailability in rats was twice that (0.028 L/min/kg) in dogs. In contrast, no difference in the distribution volumes of the three different doses given to rats was evident (range, 0.20-0.33 L/kg), and the figure was similar to that in dogs (0.28 L/kg) (Table 1). The estimated distribution volume in rats (0.374 L/kg) in the steady-state (Vss, the sum of the volumes in the central and the peripheral compartments) yielded by the compartmental model was similar to that (0.418 L/kg) in dogs. These values were similar to the sum of the plasma and extracellular fluid volumes in rats (0.328 L/kg) and dogs (0.328 L/kg), suggesting a limited distribution of SMA in tissues (Li and Corey, 2013). Although the mouse Vss (0.68 L/kg) seemed to be greater than that of other animals, both intraperitoneal bioavailability and the naïve pooled data from different test animals must be considered. The pharmacokinetic parameters estimated via model-dependent analysis were robust, as shown by re-sampling of 1,000 bootstraps, and were generally consistent with those derived via non-compartmental analysis.

Table 5 shows the predicted CL, Vss, and Vss values in a 70-kg human, derived via simple allometric scaling, using pharmacokinetic parameters estimated in mice, rats, and dogs. Please note that the rat clearance measured after administration of 30 mg/kg Tris SMA was used in allometric scaling, because higher SMA doses tended to saturate the drug metabolic and excretion systems. Log-transformed body weights correlated well with log-transformed pharmacokinetic parameters in all three species (r2>0.994, Fig. 5, 6). Mahmood and Balian developed the ‘Rule of Exponents’ to allow of a better understanding of the relationship between body weight and CL (Mahmood and Balian, 1996a; Mahmood, 2007). Simple allometry (clearance= a*weightb) predicts clearance precisely when the exponent b ranges from 0.55 to 0.7, but if the exponent ranges from 0.71 to 0.99 or 1.0 to 1.3, the MLP or brain weight should be included in modelling to better predict drug clearance in humans. As shown in Fig. 5, the exponent of the simple allometric equation was 0.9406, and the human clearance (CLhuman) was thus predicted using CL×MLP values; the final figure was divided by the human MLP (8.18×104 h) (Mahmood, 2007). Based on the brain weight (BW) of each species (Davies and Morris, 1993; Cosson et al., 1997), SMA clearance in a 70-kg human was hypothesized to be 0.25 L/min (0.08-0.78 L/min, 90% CI).

Vss and Vss in a 70-kg human were predicted to be 22.5 L (7.4-69.0 L, 95% CI) and 19.5 L (4.2-91.6 L, 90% CI) respectively, via simple allometric scaling; interspecies differences in distribution volumes are negligible once the data are normalised to body weight. The predicted human values of Vss and Vss suggest that the distribution of SMA was limited to plasma and extracellular fluid (21.2 L; Li and Corey, 2013). These findings are also comparable to those of rats and dogs. Moreover, our unpublished data revealed a tissue-to-plasma SMA concentration ratio of <1, suggesting the limited distribution into tissues.

Although the roles of hepatic and renal SMA elimination have not been clearly defined, previous studies suggest that both are important factors (Jung et al., 2014). However, the estimated systemic CLs were lower than that of hepatic blood in mice (0.04 vs. 1.31 L/min/kg, respectively), rats (0.05 vs. 1.31 L/min/kg, respectively), dogs (0.028 vs. 0.73 L/min/kg, respectively), and humans (0.51 vs. 1.3 L/min, respectively, in a 70 kg human) (Brown et al., 1997). Thus, hepatic metabolism and/or biliary excretion may contribute to the overall metabolism of SMA due to its restricted liver uptake from systemic circulation. The total clearances for different species represent a satisfactory relationship, indicating that the data obtained in this study should be sufficient to predict the human clearance of SMA.

A proposed human dose may be calculated by multiplying the clearance by the AUC associated with the desired phar-
SMA exhibited anti-tumour activity when intraperitoneally injected at 50 mg/kg into tumour-bearing mice (Regmi et al., 2015). In the present study, the AUC was 423 mg·min/L when the dose was 100 mg/kg, an AUC of 200 mg·min/L would be expected to reflect an effective dose. Consequently, a first-in-human dose of 100 mg (~1.5 mg/kg) would be appropriate, considering the expected clearance rate (0.51 L/min in a 70-kg human).

Smaller experimental animals (e.g., mice and rats) live shorter lives than do larger animals such as dogs and monkeys, and the former animals generally clear drugs more rapidly than do the latter. In contrast to chronological time, biological time differs among species, and drugs are removed at similar rates with reference to the latter type of time (Boxenbaum, 1982). The time courses of drug plasma concentrations obtained from different species may indicate that decay patterns differ over chronological time. However, if the time is transformed to pharmacokinetic (biological) time (by division by body weight and the exponents from the allometric equations yielding clearance and distribution volume), and the plasma concentrations normalised by division by dose and body weight, time courses in different animals may be superimposed, and exhibit similar decay profiles.

To explore plasma SMA concentration profiles in biological time, chronological time courses from different animals were transformed using three different species-invariant time methods: kallynochrons (elementary Dedrick plots), apolychrons (complex Dedrick plots), and dienetichrons. The data were evaluated via two-compartment modelling featuring first-order elimination, and the pharmacokinetic parameters of SMA estimated. The first-in-man dose for a 70-kg human is suggested to be 100 mg (~1.5 mg/kg). Plots of dose-normalised plasma SMA concentrations versus transformed times, from rats and dogs, were superimposable (Fig. 7), and the dienetichron plot (Fig. 7C) that considered the MLP best described the transformed dataset, compared to the kallynochron and apolychron plots, in terms of both AUCs and visual examination. The pharmacokinetic parameters (Table 6) yielded by the dienetichron plot were similar to those derived via allometric scaling.

In general, drug doses that are not associated with any observable adverse effects in experimental animals during the conduct of non-clinical toxicity studies are commonly used to estimate first-in-human doses for initial clinical trials (Food and Drug Administration, 2005); the maximum tolerable doses of anti-cancer agents may be preferred in this context (Paxton et al., 1999).

Although further work on possible SMA toxicities is under investigation, no significant adverse effect was evident following repeated 6-week intraperitoneal drug injections of 100 mg/kg (~20 mg/kg intravenous dose) into mice, where the dose was >13-fold higher than that predicted in humans. Therefore, the first-in-man dose that we suggest on the basis of efficacy of Apo2L/tumor necrosis factor-related apoptosis-inducing ligand in mice. Xenobiotica 44, 547-554.

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