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**Use of Intravenous Infusion Study Design to Simultaneously Determine Brain Penetration
and Systemic Pharmacokinetic Parameters in Rats**

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Abbreviations: AUC, area under the plasma concentration-time curve; BBB, blood-brain barrier; BSA, bovine serum albumin; CL, clearance; CNS; central nervous system; CSF, cerebrospinal fluid; C_{ss} , plasma concentration at steady-state; $f_{u,brain}$, unbound fraction in the brain; $f_{u,CSF}$,

unbound fraction in the CSF; $f_{u,p}$, unbound fraction in plasma; $K_{p,brain}$, total brain-to-plasma ratio; $K_{p,uu,brain}$, unbound brain-to-plasma ratio; $K_{p,uu,CSF}$, unbound CSF-to-plasma ratio; NCA, non-compartmental analysis; NFPS, N-[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl]sarcosine; PK, pharmacokinetics; $t_{1/2,terminal}$, terminal half-life; $t_{1/2,eff}$, effective half-life; V_{ss} , volume of distribution at steady-state.

Abstract

In drug discovery, the extent of brain penetration as measured by free brain/plasma concentration ratio ($K_{p,uu}$) is normally determined from one experiment following constant intravenous (IV) infusion, and PK parameters including clearance (CL), volume of distribution at steady-state (V_{ss}), and effective half-life ($t_{1/2,eff}$) are determined from another experiment after a single IV-bolus injection. The objective of the present study was to develop and verify a method to simultaneously determine $K_{p,uu}$ and PK parameters from a single intravenous infusion experiment. In this study, 9 compounds (atenolol, loperamide, minoxidil, NFPS, sulpiride, and 4 proprietary compounds) were intravenously infused for 4 h at 1 mg/kg or 24 h at 1 or 6 mg/kg or bolus injection at 1 mg/kg. Plasma samples were serially collected and brain and CSF samples were collected at the end of infusion. The PK parameters were obtained using non-compartmental (NCA) and compartmental analyses. The $K_{p,uu,brain}$ values of those compounds increased up to 2.86-fold from 4 h to 24 h. The CL calculated from infusion rate vs. steady-state concentration from the 24 h infusion studies was more consistent with the CL from the IV bolus studies than that from 4 h infusion studies (CL average fold-of-difference 1.19~1.44 vs. 2.10). The compartmental analysis using 1- and 2-compartment models demonstrated better performance than NCA regardless of study design. In addition, V_{ss} and $t_{1/2,eff}$ could be accurately obtained by 1-compartment analysis within 2-fold difference. In conclusion, both $K_{p,uu,brain}$ and PK parameters can be successfully estimated from a 24 h IV-infusion study design.

Keywords: Pharmacokinetics, compartmental analysis, brain penetration, intravenous infusion, drug development

Significance Statement

- We demonstrated that the extent of brain penetration and pharmacokinetic parameters (such as CL, V_{ss} and $t_{1/2,eff}$) can be determined from a single constant IV infusion study in rats.

Introduction

The blood-brain barrier (BBB) is a physiological barrier formed by brain capillary endothelial cells with tight and adherens junctions (Rubin and Staddon, 1999; Abbott et al., 2010), which contribute to protecting the brain from endogenous and exogenous toxic compounds. In drug development, BBB is the major obstacle for the drug development targeting the central nervous system (CNS). The evaluation on the extent of brain penetration of drug candidates is one of the essential steps that is conducted during drug discovery and development for CNS diseases. It is also important for non-CNS targeting drugs from a safety perspective. The brain penetration is commonly expressed as a brain partitioning coefficient or brain-to-plasma concentration ratio based on either total and unbound concentrations at steady-state ($K_{p,brain}$ and $K_{p,uu,brain}$) (Hammarlund-Udenaes et al., 2009; Reichel, 2009; Freeman et al., 2019). Since only the protein unbound drug is assumed to bind to the target (Stain-Textier et al., 1999; Bouw et al., 2000) and produce therapeutic effects, the unbound brain-to-plasma concentration ratio ($K_{p,uu,brain}$) is more physiologically relevant and widely used to describe the extent of brain penetration. Moreover, it also provides insight into the transport mechanism of a compound at BBB (Bostrom et al., 2006; Chen et al., 2014; Summerfield et al., 2016). Due to these reasons, $K_{p,uu,brain}$ is an essential factor being considered with respect to pharmacology as well as pharmacokinetics of a compound in the early drug development (Hammarlund-Udenaes et al., 2008; Hammarlund-Udenaes et al., 2009).

In general, the *in vivo* animal experiments to estimate $K_{p,uu,brain}$ and pharmacokinetic (PK) characteristics of a compound targeting CNS diseases are performed separately. For $K_{p,uu,brain}$ evaluation, 4 h intravenous (IV) infusion study using a cassette dosing is commonly used to determine $K_{p,brain}$ values of several compounds in the discovery stage/step (Friden et al., 2010;

Nagaya et al., 2016). Then, the PK characteristics of the ones with favorable brain penetration are further investigated via IV bolus injection. Although this study flow can accurately characterize the pharmacokinetics of a compound, it takes time and may not be cost-effective as two separate animal experiments are needed. If the key PK parameters of each compound can be simultaneously estimated along with $K_{p,uu,brain}$ from the same study, we can improve the efficiency for the experiment and reduce the usage of animals.

Herein, we performed IV bolus and IV-infusion studies for 4 hours (h) or 24 h to evaluate $K_{p,uu}$ of nine compounds in rats, among which five were commercially available compounds and four were proprietary compounds. Those compounds represent compounds with a wide range of CL (i.e. low, medium, and high). 1- and 2-compartment models were applied to estimate the PK parameters (e.g. CL, V_{ss} and $t_{1/2,eff}$), and the performance of the two models was evaluated as compared with the PK parameters after IV bolus injection.

Materials and Methods

Chemicals. Atenolol, loperamide, minoxidil, and sulpiride were obtained from Sigma-Aldrich (St. Louis, MO), and N-[3-(4'-fluorophenyl)-3-(4'-phenylphenoxy)propyl]sarcosine (NFPS) was purchased from Tocris Bioscience (Minneapolis, MN). Four proprietary compounds (Compound A, B, C, and D) were synthesized at Biogen. All chemicals used in the experiments were of the highest available grade.

Animal experiments. Jugular vein and carotid artery cannulated male Sprague-Dawley rats were purchased from Charles River Laboratory (Wilmington, MA). Upon arrival, the rats were acclimated for at least 3 days on a 12-hour light/dark cycle in a temperature- and humidity-controlled environment with free access to food and water. Animal experiments for proprietary

and commercial compounds were separately conducted as two cassette administrations to rats, respectively (Nagilla et al., 2011; Liu et al., 2012). For both proprietary and commercial compounds, the dosing solution was prepared by dissolving compounds with 20% captisol and filtered with 0.2 μ m syringe filter (Pall Life Sciences, Port Washington, NY). Then, compounds were intravenously injected (1 mg/kg) or infused over 4 h (1 mg/kg) or 24 h (1 and 6 mg/kg) in rats (n=3 for each group). Blood samples (100 μ L) were serially collected from the left carotid artery into EDTA containing tubes (SAI Infusion Technologies, Lake Villa, IL) at pre-determined time points after dosing. For proprietary compounds, blood samples were collected prior to dosing and at 0.083, 0.25, 0.5, 0.75, 2, 4 (last sampling for 4 h infusion group), 7, 10, 16, 22, and 24 h post-dosing. For commercial compounds, blood samples were withdrawn prior to dosing and at 0.083, 0.25, 0.5, 0.75, 1, 3, 5, 10 and 24 h after a single intravenous injection and at 0.5, 1, 2, 4 (last sampling for 4 h infusion group), 8, 16, 22, and 24 h after intravenous infusion. After blood sampling, the same volume of heparinized saline (20 IU/mL) was injected to compensate for blood loss in rats. Plasma samples were prepared after centrifugation at 10,000 rpm for 5 min. At the last sampling time, cerebrospinal fluid (CSF) samples were collected from cisterna magna at the last sampling time after CO₂ euthanasia and were immediately diluted with the equivalent volume of 8% bovine serum albumin (BSA) in PBS. Then, brain samples were harvested. The collected samples were stored at -80 °C until analysis. All animal experiments were approved by the Institutional Animal Care and Use Committee (IACUC) at Biogen, and the study was conducted in compliance with the institutional guidelines.

Sample analysis. Standard calibration curves were prepared using a serial dilution scheme of analytes in blank rat matrix. All standard calibrants were aliquoted into the extraction plate and normalized at a ratio of 1:1:1 to contain an equal mixture of plasma, brain homogenate, and

artificial cerebrospinal fluid and 8% BSA (5:5 v/v). The collected brain samples were homogenized with 2 times of volume (w/v) of PBS (pH 7.4), and 20 μ L brain homogenate was mixed with the same volume of blank plasma and mixture of artificial cerebrospinal fluid and 8% BSA (5:5 v/v). For plasma sample preparation, 20 μ L of plasma sample was mixed with the same volume of brain homogenate and blank mixture of artificial cerebrospinal fluid and 8% BSA (5:5 v/v). For CSF sample preparation, 20 μ L of CSF sample was added into 20 μ L of each blank plasma and brain homogenate. Then, proteins in a total of 60 μ L of the standard, matrix blanks or a sample was precipitated with 360 μ L of acetonitrile or acetonitrile containing internal standards (glyburide, carbutamide, and chrysin). After vortexing and centrifuging at 3,500 rpm for 10 minutes, 250 μ L of supernatant was transferred into a 96-well injection plate and dried under nitrogen gas at 40 °C. Then, samples were reconstituted with 100 μ L the mixture of water and acetonitrile (50:50 v/v) and analyzed with high-performance liquid chromatography equipped with mass spectrometry (Triple QuadTM 5500 System, AB Sciex, Framingham, MA). Mobile phases used were 0.1% formic acid in water and 0.1% formic acid in acetonitrile along with an Ace EXCEL 3 C18-PFP 2.1 \times 50 mm column (3 μ m particle size; Advanced Chromatography Technologies Ltd., Aberdeen, Scotland).

Single intravenous bolus injection data analysis. Noncompartmental analysis (NCA) was applied to estimate PK parameters from the intravenous bolus injection data. PK parameters including clearance (CL), volume of distribution at steady-state (V_{ss}), dose-normalized area under the plasma concentration-time curve ($AUC_{\infty}/Dose$) and terminal elimination half-life ($t_{1/2,terminal}$) were estimated by Phoenix[®] WinNonlin[®] (version 7.0; Pharsight Corporation, Cary, NC). The effective half-life ($t_{1/2,eff}$) was calculated using Equation 1. It was proposed to reflect drug accumulation after multiple doses (Boxenbaum and Battle, 1995) while $t_{1/2,terminal}$ is a

dependent parameter upon elimination phase. This treatment is a simplification of a more complex pharmacokinetic process in drug discovery where $t_{1/2}$ is estimated from predicted CL and $V_{d_{ss}}$.

$$t_{1/2,eff} = \frac{\ln 2 \times V_{ss}}{CL} \quad (\text{Equation 1})$$

Intravenous infusion data analysis. Total ($K_{p,brain}$) and unbound ($K_{p,uu,brain}$) brain to plasma partition coefficients as well as the unbound CSF to plasma ratio ($K_{p,uu,CSF}$) were calculated as follows:

$$K_{p,brain} = \frac{C_{brain}}{C_p} \quad (\text{Equation 2})$$

$$K_{p,uu,brain} = \frac{C_{brain} \cdot f_{u,brain}}{C_p \cdot f_{u,p}} \quad (\text{Equation 3})$$

$$K_{p,uu,CSF} = \frac{C_{CSF} \cdot f_{u,CSF}}{C_p \cdot f_{u,p}} \quad (\text{Equation 4})$$

C_{brain} , C_p and C_{CSF} are total brain, plasma, and CSF concentrations at the end of infusion, respectively, and $f_{u,brain}$ and $f_{u,p}$ are unbound fractions in the brain and plasma, respectively. The unbound fraction in the CSF ($f_{u,CSF}$) was calculated from $f_{u,p}$ using a single binding site model as follows (Friden et al., 2009):

$$f_{u,CSF} = \frac{1}{1 + Q_{alb} \left(\frac{1}{1 - f_{u,p}} - 1 \right)} \quad (\text{Equation 5})$$

Q_{alb} is the ratio of albumin in CSF over that in plasma, which was set to 0.003 for rats (Habgood et al., 1992).

Noncompartmental analysis was applied to determine CL from 4 h and 24 h infusion data using the following equation:

$$CL = \frac{\text{Infusion rate}}{C_{ss}} \quad (\text{Equation 6})$$

The plasma concentration at steady-state (C_{ss}) was defined as the plasma concentration at 4 h for the 4 h infusion study and average plasma concentration of 22 h and 24 h for the 24 h infusion study. In addition, the compartmental analysis was performed using both 1- and 2-compartment models, which were incorporated in Phoenix[®] WinNonlin[®] (version 7.0; Pharsight Corporation, Cary, NC), to estimate the PK parameters, particularly for V_{ss} which cannot be obtained via noncompartmental analysis in this study. Using the obtained CL and V_{ss} , the effective half-life ($t_{1/2,eff}$) was calculated using Equation 1 (Gunaydin et al., 2018; Smith et al., 2018), as the terminal half-life ($t_{1/2,terminal}$) could not be estimated due to the lack of elimination phase of the infusion data.

The PK parameters were shown as mean \pm S.D. The one-way ANOVA with post hoc Tukey's test was performed to compare $K_{p,brain}$ or $K_{p,uu,brain}$ values among different study designs using GraphPad Prism (ver. 8.3.0; San Diego, CA), and p values < 0.05 were considered statistically significant. Moreover, the estimated CL, V_{ss} , and $t_{1/2,eff}$ values of each compound from constant infusion studies were divided by those from bolus injection to obtain the fold difference to compare the performance of different study designs as well as the data analysis methods. The average fold-difference was calculated to assess the performance of different estimation approaches. Simple linear regression was performed to seek correlations of the calculated PK parameters after a single IV injection with the estimated PK parameters by NCA and/or compartmental analyses after IV infusion. The correlations plots for CL, V_{ss} , and $t_{1/2,eff}$ were depicted in Supplementary Figures S1~S3, respectively.

Results

Pharmacokinetics following a single IV injection. The plasma concentrations vs. time profiles of the proprietary (4 compounds) and commercial compounds (5 compounds) after the single IV injection were depicted in **Figure 1**, and the estimated PK parameters for each compound were shown in **Table 1**. Most of the compounds exhibited lower CLs than the hepatic blood flow rate (55 mL/min/kg) (Davies and Morris, 1993), except for minoxidil (113 mL/min/kg) and sulpiride (82.9 mL/min/kg). For V_{ss} , all of the test compounds were higher than 1 L/kg (Kwon, 2001) although some had large variations in V_{ss} possibly due to a small sample size. The terminal phase half-lives ($t_{1/2,terminal}$ s) ranged between 1.89 h and 48.9 h, whereas the effective half-lives ($t_{1/2,eff}$ s) were relatively smaller than $t_{1/2,terminal}$ s for which the values were between 0.79 h and 15.1 h (**Table 1**). The fractions of the unbound plasma and brain protein binding ($f_{u,p}$ and $f_{u,brain}$) for each compound were collected from the literature or generated in house (**Table 1**) (Liu et al., 2005; Kodaira et al., 2011; Srikanth et al., 2013; Liu et al., 2018). Six of the nine compounds were highly bound to proteins in plasma and brain ($f_{u,p}$ and $f_{u,brain} < 0.1$). Among the other three compounds, the $f_{u,p}$ and $f_{u,brain}$ values were 0.135 and 0.0592 for Compound B and 0.685 and 0.658 for minoxidil, respectively, and atenolol showed the lowest protein binding among the tested compounds in both plasma and brain (**Table 1**). The $f_{u,CSF}$ values for all of the test compounds, calculated by equation 5, were higher than 0.9706, indicating most of compounds in CSF presented in CSF as unbound forms.

Brain penetration of the compounds: $K_{p,uu,brain}$ at 4 h is lower than $K_{p,uu,brain}$ at 24 h for most compounds. The $K_{p,brain}$, $K_{p,uu,brain}$, and $K_{p,uu,CSF}$ values for each compound were calculated using Equations 2, 3, and 4, respectively (**Table 2**). Overall, both $K_{p,brain}$ and $K_{p,uu,brain}$ values after 4 h IV infusion were lower compared with the values in the 24 h IV infusion studies, except Compound B and loperamide whose $K_{p,brain}$ and $K_{p,uu,brain}$ values were similar in both 4 h and 24

h infusion studies. In particular, notable increases in $K_{p,brain}$ and $K_{p,uu,brain}$ values of Compound C, NFPS and sulpiride were observed by prolonged infusion time from 4 h to 24 h (**Table 2**). Despite the same infusion rate of 1 mg/kg/4 h and 6 mg/kg/24 h infusion studies, the calculated $K_{p,brain}$ and $K_{p,uu,brain}$ for Compound C, NFPS and sulpiride in 24 h infusion groups were 70, 224 and 73% higher than those in 4 h infusion groups, respectively. Interestingly, the compounds with a longer half-life (> 4 h) such as NFPS exhibited a significant increase in brain penetration ($K_{p,uu,brain}$) by prolonged infusion time. $K_{p,brain}$ and $K_{p,uu,brain}$ values of the Compound A, C, and D were slightly increased by 1.20 ~ 1.70-fold after 24 h infusion, and there were no differences in $K_{p,brain}$ and $K_{p,uu,brain}$ of Compound B and atenolol between 4 h and 24 h infusion studies.

The CSF samples were only collected from the studies with the commercial compounds. Some of the determined CSF concentrations of loperamide, minoxidil and NFPS were below the limit of quantification. Therefore, $K_{p,uu,CSF}$ for those compounds could not be calculated or were shown without standard deviations ($n=1\sim2$) (**Table 2**). The average fold-differences between $K_{p,uu,CSF}$ and $K_{p,uu,brain}$ for atenolol, minoxidil and sulpiride were about within 2 folds, while loperamide and NFPS showed substantial differences between $K_{p,uu,CSF}$ and $K_{p,uu,brain}$. The observed $K_{p,uu,brain}$ and $K_{p,uu,CSF}$ of commercial compounds were comparable with the reported values in the literature, except sulpiride (**Table 3**).

CL from IV infusion studies: 24 h-infusion studies provided more accurate CL than the 4 h-infusion studies. The CLs were calculated using NCA with equation $CL = \text{infusion rate}/C_{ss}$ (Equation 6), and PK parameters, including CL, V_{ss} , and $t_{1/2,eff}$ were estimated using compartmental analyses, then normalized to the PK parameters determined from IV injection data for each compound (**Tables 4-6**). The observed data and the fitted results by 1- and 2-compartment models based on infusion data were depicted in **Figures 2~4**. Overall, most of the

estimated CLs from the constant IV infusion data by both non-compartmental and compartmental analyses were within 2-fold difference compared to those of the single IV bolus data, while NCA method based on 4 h-infusion data slightly overestimated CL by more than 2 folds (**Table 4**). The average fold-difference of CL in the 24 h-infusion groups normalized to the CL data of the IV bolus groups were much closer to 1 than that in the 4 h-infusion groups regardless of non-compartmental and compartmental analyses (**Table 4**). The CL tends to be over-estimated in the 4 h infusion studies probably due to not having enough time for these compounds to reach the steady states *in vivo*. The average fold-differences of the CL values determined by 1- and 2-compartment models in the 24 h-infusion studies ranged between 1.07~1.42 and 1.13~1.29, respectively, while the average fold-differences of the CL values determined by both models in the 4 h-infusion studies were between 1.81~1.82, suggesting that the 24 h-infusion studies provided more accurate CL than the 4 h-infusion studies (**Table 4**). In particular, the calculated CL of Compound D in the 4 h-infusion study by NCA was 4.48-fold higher than the actual CL obtained after single IV bolus injection, while the average fold-differences were less than 1.40 in the 24 h-infusion studies (estimated by the same NCA approach) (**Table 4**).

With respect to the methodology for CL estimation, the average fold-difference of CLs estimated by NCA and 1- and 2-compartment models were 2.10, 1.81 and 1.82 for 1 mg/kg/4 h study, 1.44, 1.42, and 1.29 for 1 mg/kg/24 h study, and 1.19, 1.07, and 1.13 for 6 mg/kg/24 h study, respectively (**Table 4**), indicating that NCA provided the most inaccurate CLs among the estimation methods (NCA, 1- and 2-compartmental analyses), regardless of the study design. In comparison, the observed data were well described by both 1- and 2-compartment models (**Figures 2-4**), and the estimated CLs by both models were similar and more accurate than the

values by NCA. The average fold-differences of the CL values determined by 1- and 2-compartment models in the 4 h- and 24 h-infusion studies ranged between 1.07~1.81 and 1.13~1.82, respectively, while the average fold-differences of the CL values determined by NCA in the 4 h- and 24 h-infusion studies were between 1.19~2.10, suggesting that the compartmental analyses provided more accurate CL than NCA (**Table 4**).

V_{ss} from IV infusion study: the 24 h-infusion studies provided more accurate V_{ss} than 4 h-infusion studies. The compartmental analyses using 1- and 2-compartment models were applied to estimate V_{ss} from the 4 h- and 24 h-infusion studies. The average fold-differences of the V_{ss} values were within 2 folds between the 4 h- and 24 h-infusion studies, except for the estimated V_{ss} by 2-compartment model based on the 1 mg/kg/24 h infusion studies (**Table 5**). The estimated V_{ss} from the 4 h infusion studies tended to be underestimated by both 1- and 2-compartment models compared with that in the IV bolus studies; the average fold-differences for 1- and 2-compartment models were 0.58 and 0.79, respectively. Interestingly, the 24 h infusion studies provided more accurate V_{ss} than 4 h infusion studies.

Application of compartmental analyses for $t_{1/2,eff}$ estimation from IV infusion study without elimination phase showed the average fold-differences were close to 1 fold or within 2 folds of the actual values. The effective half-life ($t_{1/2,eff}$) was indirectly derived from the estimated CL and V_{ss} using Equation 1. Therefore, only the values from the compartmental analyses using 1- and 2-compartment models were used to derive $t_{1/2,eff}$ (**Table 6**). Among the tested compounds, the $t_{1/2,eff}$ of Compound D was poorly predicted in the 4 h-infusion study, as the calculated $t_{1/2,eff}$ values by 1- and 2-compartment models were 15% and 11% of the actual values. For most of other compounds, the average fold-differences in the $t_{1/2,eff}$ were very close to 1 fold or within 2 folds of the actual value (**Table 6**). Overall, using CL and V_{ss} estimated by 1-compartment

model showed slightly better performance in predicting $t_{1/2,eff}$ than using the estimates by 2-compartment model (**Table 6**).

Discussion

Brain penetration of compounds is a highly essential element in the drug discovery stage for CNS diseases. Thus, animal experiments should be adequately designed to accurately determine $K_{p,uu,brain}$ of the tested compounds with various and different physicochemical properties. Although a cassette dosing with IV infusion for 4 h is typically conducted to determine $K_{p,uu,brain}$ in rats (Friden et al., 2010; Nagaya et al., 2016), 4 h-infusion may not be long enough to reach the steady-state for $K_{p,uu,brain}$ evaluation, if the compound has a longer half-life (Zheng, 2015). Thus, the longer duration of infusion is necessary for the compounds to reach equilibrium in plasma and brain, as demonstrated by the current study.

Comparing $K_{p,brain}$ and $K_{p,uu,brain}$ values from the 4 h- and 24 h-infusion studies with the same infusion rate (1 mg/kg/4 h vs. 6 mg/kg/24 h), the overall values of the 24 h-infusion studies tended to be higher than those of the 4 h-infusion studies (**Table 2**), indicating that 4 h-infusion was not sufficient to reach steady-state. In particular, the brain penetration ($K_{p,uu,brain}$) of Compound C and NFPS following 6 mg/kg/24 h infusion were 1.71- and 2.14-fold higher than the values after 1 mg/kg/4 h infusion, respectively (**Tables 2**). When taking into consideration that $t_{1/2,terminal}$ values of Compound C and NFPS were 6.8 h and 4.59 h (**Table 1**), the study design with 4 h infusion could not be adequate to evaluate the brain penetration of a compound with a long half-life. Although most of the obtained $K_{p,uu,brain}$ and $K_{p,uu,CSF}$ for the commercial compounds were comparable with the previously reported values, considerable differences were found in those values of sulpiride (**Tables 2 and 3**). As sulpiride is not a substrate of P-gp and

BCRP (**Table 3**), $K_{p,uu,brain}$ (1.11~1.93) and $K_{p,uu,CSF}$ (2.57~3.33) in this study might be more reliable than the values in the literature (< 0.05) (Kodaira et al., 2011). In addition, the substantial difference between $K_{p,uu,brain}$ and $K_{p,uu,CSF}$ for loperamide and NFPS suggested that $K_{p,uu,CSF}$ or CSF concentration cannot be utilized as a surrogate marker reflecting unbound brain concentration of the compounds with poor brain penetration (Lin, 2008).

The modified study design with serial blood sampling during the infusion allowed to estimate critical PK parameters of a compound such as CL, V_{ss} , and $t_{1/2,eff}$ simultaneously in addition to the determination of $K_{p,uu}$. In the present study, we demonstrated that 4 h-infusion was not adequate to obtain accurate PK parameters, particularly for CL and V_{ss} , and 24 h-infusion is more accurate than 4 h on PK parameter determination. For CL, the average fold-difference of the 4 h infusion groups indicated that CL was overestimated by both NCA (2.1-fold) and compartment analyses (1.82-fold). In particular, the calculated CLs of Compound D following 4 h infusion was 4.48-fold higher than the actual CL after IV bolus injection estimated by NCA, while a longer infusion time provided more accurate CL (1.23~1.40-fold; **Table 4**). For V_{ss} , the estimated values by both 1- and 2-compartment models from the 4 h-infusion studies tended to be underestimated, possibly due to the overestimated CLs when considering the inverse association between CL and volume of distribution (**Tables 4 and 5**). For the same reason, studies with 24 h constant infusions exhibited better performance in estimating V_{ss} (**Table 5**). These results suggested that 24 h infusion is a more appropriate study design for both $K_{p,uu,brain}$ and PK parameter estimation. Furthermore, the average fold-differences indicated that the PK parameters from both 1 mg/kg/24 h and 6 mg/kg/24 h infusion groups were similar and more accurate compared with the values of 1 mg/kg/4 h infusion groups (**Tables 4~6**), suggesting that infusion time is more critical for PK parameter estimation than infusion rate. We also

demonstrated that compartment analysis is a useful approach to obtain PK parameters from a constant IV infusion study without elimination phase. Although the steady-state of Compound D was not achieved in the 4 h-infusion studies, compartment analyses using 1- and 2-compartment models provided more accurate CL values than NCA (**Table 4**). When 1- and 2-compartment models were applied to the 24 h-infusion studies (1 and 6 mg/kg), the average fold-differences in CL and V_{ss} by 1- and 2-compartment models were similar with acceptable accuracy; 1.07~1.42 and 1.13~1.29 for CL and 0.79~0.82 and 0.97~1.10 for V_{ss} (without loperamide), respectively (**Tables 4 and 5**). Similarly, $t_{1/2,eff}$ was also more accurately estimated by 1-compartment model than 2-compartment model when compared average fold differences of 24 h infusion studies in **Table 6**. Comparable performances of 1- and 2-compartment models in this study suggest that 1-compartment model should be sufficient to obtain PK parameters from 24 h constant infusion studies with no elimination phases in terms of model simplicity.

Infusion over 24 h could be technically challenging, sometimes depending on the physiochemical property of the compound (e.g. solubility of the compound). A formulation is generally needed which is stable over this time interval and physiologically acceptable for dosing in terms of volume and composition. Solubility (in aqueous solution) and stability of a compound are generally determined and optimized by chemist and formulation scientist prior to in vivo study, while it could still be challenging and not feasible to find the best combination of formulations for all the compounds. The best practice was applied, and dosing solutions were filtered prior to IV infusion. In addition, to avoid overestimated PK parameters caused by the compound's poor solubility, the actual drug concentrations of the dosing solution were also measured with the above-mentioned method and then used for further PK analyses.

As presented, we validated that both brain penetration and informative PK parameters of a compound could be successfully estimated by applying compartmental analysis to constant infusion studies. In drug development, the pharmacokinetics of a compound is generally determined in a separate study after testing brain penetration, due to the nature of differences in the study designs. Very few studies have been reported in an effort to consolidate two different studies into one study. Bridges et al. (2014) developed a study design with single IV bolus dosing of compounds as a cassette dosing followed by another single IV bolus injection at 24 h after the first dosing. The brain was then harvested at 15 min after the second dosing. However, this method does not assure whether the steady-state is achieved, leading to underestimation of $K_{p,uu,brain}$ for a compound, particularly with low permeability. Fu and colleagues (Fu et al., 2018) established another study design with a single oral administration followed by IV infusion for 17 h to evaluate bioavailability, $K_{p,uu,brain}$, and CL from one study. Although this approach allowed to evaluate bioavailability with brain penetration of the target compound, V_{ss} could not be obtained from the study. Furthermore, both approaches by Bridges et al. (2014) and Fu et al. (2018) eventually had two different studies conducted sequentially, not consolidating 2 different studies into 1 study, as the brain penetration and the PK parameters of a compound were separately derived from two different studies that were performed in the same animals. In this study, we established a novel approach to evaluate brain penetration as well as critical PK parameters of the tested compounds by using compartmental analysis in one study with 24 h constant infusion. According to the study by Jusko and Gibaldi (1972), about 90% of steady-state could be achieved when a drug is infused for > 3 mean residence time (MRT). Fu et al. (2018) reported that 88% of the compounds in the internal database (> 30,000 compounds) had a shorter MRT than 5 h, inferring 24 hours of IV infusion utilized in the current study could be enough to

reach steady-state for most of the compounds in early drug discovery. Moreover, another strength of the 24 h infusion study design is that it enables the assessment of V_{ss} from a constant IV infusion study with frequent sampling during infusion. Although a drug with low V_{ss} (< 0.6 L/kg) (Smith et al., 2015) was not tested in this study, simulation proved that this study design is applicable to estimate V_{ss} of compounds with wide range of V_{ss} (**Figure 5**).

However, the limitation of the current study is that the study design does not allow to estimate bioavailability of test compound, that is also an important aspect to be considered in drug development. Although an additional in vivo study is required to evaluate the bioavailability of compounds, the infusion study enables the narrowing down of compounds which could be further investigated. In other words, time and resources can be saved by performing bioavailability test for the optimal compounds with favorable brain penetration, CL, V_{ss} , and $t_{1/2}$. Further investigation is needed to develop a more efficient study design or PK approach for estimation of bioavailability along with other PK parameters (e.g. CL, V_{ss} , and $t_{1/2}$) as well as brain penetration.

In summary, we developed and validated a method to determine not only $K_{p,uu,brain}$ but also PK parameters from one single 24 h IV-infusion study design.

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Author Contributions

Participated in study design: Liu, Wei

Conducted experiments: Noh, Pietrasiewicz

Performed data analysis: Noh

Wrote and contributed to the writing of the manuscript: Noh, Liu, Wei

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Figure legends

Figure 1. Plasma concentration vs. time profiles of (A-D) internal and (E-I) commercial compounds in rats after single intravenous injection as a cassette dosing (1 mg/kg). The closed circles represent observed data (n=3; mean \pm S.D).

Figure 2. Plasma concentration vs. time profiles of (A-D) internal and (E-I) commercial compounds in rats following a constant intravenous infusion over 4 h as a cassette dosing (1 mg/kg). The closed circles represent observed data (n=3; mean \pm S.D), and the fitted results by 1- and 2-compartment models are depicted as solid and dashed lines, respectively.

Figure 3. Plasma concentration vs. time profiles of (A-D) internal and (E-I) commercial compounds in rats following a constant intravenous infusion over 24 h as a cassette dosing (1 mg/kg). The closed circles represent observed data (n=3; mean \pm S.D), and the fitted results by 1- and 2-compartment models are depicted as solid and dashed lines, respectively.

Figure 4. Plasma concentration vs. time profiles of (A-D) internal and (E-I) commercial compounds in rats following a constant intravenous infusion over 24 h as a cassette dosing (6 mg/kg). The closed circles represent observed data (n=3; mean \pm S.D), and the fitted results by 1- and 2-compartment models are depicted as solid and dashed lines, respectively.

Figure 5. Simulated plasma concentration vs. time profiles of compounds with low, moderate and high volume of distribution after a constant intravenous infusion over 24 h. The V_{ss} values were set to 0.1, 0.3, 0.6, 1, 5, and 10 L/kg, and the CLs values were assumed to be the same as the hepatic blood flow rate (55 mL/min/kg). For simulation, it was assumed that the blood samples were serially collected at 0.083, 0.25, 0.5, 0.75, 1, 3, 5, 10 and 24 h after infusion.

Table 1. Pharmacokinetic parameters calculated by noncompartmental analysis (NCA) after 1 mg/kg single intravenous injection of compounds (n=3 for each group).

	Internal compounds				Commercial compounds				
	A	B	C	D	Atenolol	Loperamide	Minoxidil	NFPS	Sulpiride
CL (mL/min/kg)	28.0 ± 3.28	31.3 ± 7.95	7.44 ± 2.07	3.29 ± 0.91	35.5 ± 2.56	22.5 ± 4.24	113 ± 17.2	18.4 ± 2.05	82.9 ± 8.86
V _{ss} (L/kg)	2.80 ± 0.30	11.5 ± 15.5	1.42 ± 0.25	3.99 ± 0.94	5.56 ± 1.03	2.72 ± 0.47	6.96 ± 6.93	7.07 ± 0.69	8.25 ± 2.31
AUC _{inf} /Dose (ng·h/mL/mg/kg)	600 ± 69.2	560 ± 166	2350 ± 580	5330 ± 1430	471 ± 32.7	757 ± 131	149 ± 23.1	1912 ± 95.6	204 ± 22.5
t _{1/2,terminal} (h)	3.46 ± 1.73	48.9 ± 72.0	6.80 ± 6.58	14.9 ± 8.65	3.71 ± 1.53	2.14 ± 0.78	4.53 ± 7.15	4.59 ± 0.22	1.89 ± 1.18
t _{1/2,eff} (h)	1.16 ± 0.06	5.65 ± 8.38	2.25 ± 0.30	15.1 ± 7.07	1.83 ± 0.45	1.43 ± 0.41	0.79 ± 0.88	4.44 ± 0.13	1.18 ± 0.45
f _{u,p}	0.00375 ^a	0.135 ^a	0.0131 ^a	0.0266 ^a	0.91 ^b	0.0701 ^c	0.640 ^d	0.041 ^e	0.0347 ^c
f _{u,brain}	0.00613 ^a	0.0592 ^a	0.0106 ^a	0.00402 ^a	0.599 ^d	0.00196 ^c	0.658 ^d	0.0017 ^e	0.345 ^c
f _{u,csf} ^f	1.0	0.9995	1.0	0.9999	0.9706	0.9998	0.9947	0.9999	0.9999

Data were shown as mean ± S.D.

^a Data were obtained from the internal database.

^b obtained from Srikanth et al. (2013)

^c obtained from Kodaira et al. (2011)

^d obtained from Liu et al. (2018). For minoxidil, it was assumed that unbound fractions in blood and plasma are the same.

^e obtained from Liu et al. (2005)

^f calculated by Equation 5 (Frider et al., 2009)

Table 2. Calculated total ($K_{p,brain}$) and unbound ($K_{p,uu,brain}$) brain- and unbound CSF ($K_{p,uu,CSF}$)-to-plasma ratios.

	Internal compounds				Commercial compounds				
	A	B	C	D	Atenolol	Loperamide	Minoxidil	NFPS	Sulpiride
Total brain-to-plasma ratio ($K_{p,brain}$)									
1 mg/kg over 4 h	0.49 ± 0.14	0.23 ± 0.06	0.30 ± 0.05	3.11 ± 0.44	0.08 ± 0.01	0.15 ± 0.03	0.14 ± 0.03	0.33 ± 0.04	0.11 ± 0.01
1 mg/kg over 24 h	0.72 ± 0.12	0.21 ± 0.07	0.47 ± 0.05	4.04 ± 0.68	0.09 ± 0.01	0.10 ± 0.02	0.29 ± 0.17	0.01 ± 0.38*	0.18 ± 0.01
6 mg/kg over 24 h	0.81 ± 0.14	0.28 ± 0.06	0.51 ± 0.10*	3.73 ± 0.54	0.09 ± 0.02	0.15 ± 0.04	0.19 ± 0.08	0.74 ± 0.18	0.19 ± 0.05*
Unbound brain-to-plasma ratio ($K_{p,uu,brain}$) ^a									
1 mg/kg over 4 h	0.80 ± 0.23	0.10 ± 0.03	0.24 ± 0.04	0.47 ± 0.07	0.05 ± 0.01	0.004 ± 0.001	0.14 ± 0.03	0.014 ± 0.001	1.11 ± 0.08
1 mg/kg over 24 h	1.17 ± 0.20	0.09 ± 0.03	0.38 ± 0.04	0.61 ± 0.10	0.06 ± 0.004	0.003 ± 0.001	0.29 ± 0.17	0.04 ± 0.02*	1.84 ± 0.13*
6 mg/kg over 24 h	1.32 ± 0.23	0.12 ± 0.03	0.41 ± 0.08*	0.56 ± 0.08	0.06 ± 0.01	0.004 ± 0.001	0.20 ± 0.09	0.03 ± 0.01	1.93 ± 0.53*
Unbound CSF-to-unbound plasma ratio ($K_{p,uu,CSF}$) ^b									
1 mg/kg over 4 h	N.A.	N.A.	N.A.	N.A.	0.094 ± 0.044	0.318 ± 0.143	0.14 ± 0.02	0.253 ± 0.088	2.57 ± 0.03
1 mg/kg over 24 h	N.A.	N.A.	N.A.	N.A.	0.091 ± 0.058	0.451 ^c	0.17 ^c	0.412 ^c	3.33 ± 1.69
6 mg/kg over 24 h	N.A.	N.A.	N.A.	N.A.	0.081 ± 0.018	0.126 ^c	0.16 ± 0.05	0.169 ± 0.057	3.05 ± 0.36

^a LogP values for internal and commercial compounds except for minoxidil were calculated by using ChemDraw Professional 16 (PerkinElmer Informatics, Inc). LogP value for minoxidil was obtained from PubChem.

^a Unbound brain-to-plasma ratio was calculated using Equation 3, and unbound fractions in plasma and brain were shown in Table 1.

^b CSF-to-unbound plasma ratio was calculated using Equation 4, and unbound fractions in plasma were shown in Table 1.

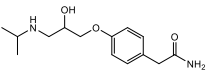
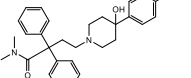
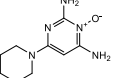
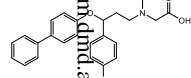
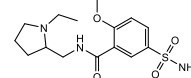
^c S.D. was not available, as the sample size was less than 3.

N.A.: not available as CSF samples were not collected

N.C.: not calculated as drug concentration in CSF was not detectable.

* $p < 0.05$ compared with 1 mg/kg infusion over 4 h

Table 3. Physicochemical and PK properties of test compounds from internal database and literature.

	Internal compounds				Commercial compounds				
	A	B	C	D	Atenolol	Loperamide	Minoxidil	IFPS	Sulpiride
Structure	N.A.	N.A.	N.A.	N.A.					
Physicochemical properties									
LogP ^a	6.28	1.38	5.7	2.89	0.5	5.15	1.24	1.83	0.43
LogS ^b	-6.66	-3.25	-6.14	-4.96	-1.50	-6.49	-2.84	5.92	-2.81
PK properties (<i>in vivo</i>)									
Hepatic extraction ratio (E _H)	0.707 ^c	0.349 ^c	0.088 ^c	0.037 ^c	0.3 ^e	N.A.	N.A.	N.A.	N.A.
CL (mL/min/kg)	N.A.	N.A.	N.A.	N.A.	38.9 ^f 24.5 ^g	128 ⁿ	N.A.	13 ^r	41.0 ^t
V _{ss} (L/kg)	N.A.	N.A.	N.A.	N.A.	7.02 ^f 2.88 ^g	9.0 ⁿ	N.A.	N.A.	2.11 ^t
AUC _{inf} /Dose (ng·h/mL/mg/kg)	N.A.	N.A.	N.A.	N.A.	789 ^h 765 ⁱ	131 ⁿ	N.A.	N.A.	402 ^t
t _{1/2,terminal} (h)	N.A.	N.A.	N.A.	N.A.	2.21 ^f 2.76 ^g	1.0 ⁿ	N.A.	2.3 ^r	0.94 ^t
K _{p,uu,brain}	N.A.	N.A.	N.A.	N.A.	0.026 ^j 0.034 ^k	0.00886 ^o	0.175 ^k	0.018 ^s 0.12 ^s	0.0219 ^o
K _{p,uu,CSF}	N.A.	N.A.	N.A.	N.A.	0.036 ^j	0.0376 ^o	N.A.	0.13 ^s	0.0499 ^o
PK properties (<i>in vitro</i>)									
Efflux ratio (MDCK-MDR1)	2.16 ^d	5.68 ^d	1.40 ^d	0.865 ^d	0.416 ^g 0.69 ^l 2.33 ^m	17.8 ^m 212 ^p	1.3 ^p 3 ^q	N.A.	0.9 ^u
Efflux ratio (MDCK-BCRP)	1.30 ^d	21.0 ^d	0.548 ^d	0.46 ^d	1.43 ^l	27.0 ^d	4.2 ^d	N.A.	0.76 ^o

N.A.: not available

^a LogP values for internal and commercial compounds except for minoxidil were calculated by using ChemDraw Professional 16 (PerkinElmer Informatics, Inc). LogP value for minoxidil was obtained from PubChem.

^b LogS values for internal and commercial compounds were calculated by using ChemDraw Professional 16 (PerkinElmer Informatics, Inc).

^c Hepatic extraction ratio or E_H for internal compounds was calculated by hepatic clearance (CL_H)/ hepatic blood flow (Q_H). The Q_H was set to 55 mL/min/kg (Davies and Morris, 1993), and CL_H was obtained from rat hepatocytes stability test of internal database.

- ^d Data were obtained from the internal database.
- ^e Data were obtained from Hung et al. (2001).
- ^f Data were obtained from Belpaire et al. (1990).
- ^g Data were obtained from Chen et al. (2020).
- ^h Data were obtained from Lemmer et al. (1985).
- ⁱ Data were obtained from Mehvar et al. (1990).
- ^j Data were obtained from Friden et al. (2009).
- ^k Data were obtained from Liu et al. (2018).
- ^l Data were obtained from Bicker et al. (2017).
- ^m Data were obtained from Hellinger et al. (2012).
- ⁿ Data were obtained from Zamek-Gliszczynski et al. (2012).
- ^o Data were obtained from Kodaira et al. (2011). The efflux ratio of sulpiride was obtained by using mouse Bcrp-transfected MDC.
- ^p Data were obtained from Li et al. (2013).
- ^q Data were obtained from Nagar et al. (2014).
- ^r Data were obtained from Liu et al. (2005) after subcutaneous injection. The bioavailability was assumed to be 100%.
- ^s Data were obtained from Liu et al. (2006).
- ^t Data were obtained from Yamada et al. (1990).
- ^u Data were obtained from Feng et al. (2008).

Table 4. CLs estimated by non-compartmental and compartmental analyses based on 4 h and 24 h intravenous infusion studies. Each value was normalized to the CL of IV bolus data, then shown as average fold-difference. The actual mean CL values for each group were shown in the bracket (mL/min/kg).

	Internal compounds				Commercial compounds					Total	
	A	B	C	D	Atenolol	Loperamide	Minoxidil	NFPS	Suppiride	Average	CV%
Noncompartmental analysis (NCA)											
IV bolus	1 (28.0)	1 (31.3)	1 (7.44)	1 (3.29)	1 (35.5)	1 (22.5)	1 (113.4)	1 (18.4)	1 (22.9)	1	0
Noncompartmental analysis (NCA)											
1 mg/kg over 4 h	0.81 (22.6)	1.12 (35.1)	1.22 (9.10)	4.48 (14.7)	1.61 (57.3)	4.55 (103)	0.99 (113)	2.78 (51.1)	1.30 (29.08)	2.10	70.8
1 mg/kg over 24 h	0.71 (19.9)	2.49 (77.9)	0.76 (5.66)	1.40 (4.62)	1.27 (45.0)	3.10 (69.9)	0.90 (101.6)	1.22 (22.4)	1.15 (25.0)	1.44	56.4
6 mg/kg over 24 h	0.85 (23.9)	2.05 (64.1)	1.02 (7.61)	1.26 (4.14)	0.65 (23.0)	3.03 (68.3)	0.62 (70.4)	0.66 (12.1)	0.62 (11.1)	1.19	69.3
1-compartment analysis (1-comp)											
1 mg/kg over 4 h	0.57 (16.1)	0.99 (31.0)	0.61 (4.58)	3.57 (11.7)	1.66 (59.0)	4.07 (91.8)	1.02 (116)	2.56 (47.1)	1.29 (30.7)	1.81	71.0
1 mg/kg over 24 h	0.73 (20.5)	2.29 (71.7)	0.83 (6.20)	1.21 (3.98)	1.23 (43.7)	3.29 (74.2)	0.90 (102.5)	1.16 (21.4)	1.11 (21.7)	1.42	58.9
6 mg/kg over 24 h	0.89 (25.0)	1.42 (44.4)	1.01 (7.54)	0.86 (2.84)	0.68 (24.0)	2.91 (65.6)	0.61 (68.8)	0.61 (11.1)	0.62 (51.6)	1.07	69.2
2-compartment analysis (2-comp)											
1 mg/kg over 4 h	0.57 (16.0)	0.99 (31.0)	0.61 (4.54)	3.92 (12.9)	1.53 (54.3)	4.07 (91.8)	0.89 (101)	2.55 (47.0)	1.27 (106)	1.82	75.1
1 mg/kg over 24 h	0.70 (19.7)	2.14 (66.9)	0.62 (4.60)	0.87 (2.88)	1.23 (43.6)	2.87 (64.6)	0.90 (102.4)	1.16 (21.3)	1.11 (91.7)	1.29	57.4
6 mg/kg over 24 h	0.83 (23.2)	1.42 (44.4)	0.98 (7.30)	0.64 (2.09)	0.66 (23.4)	3.85 (86.9)	0.60 (68.1)	0.60 (11.1)	0.61 (50.8)	1.13	93.1

Table 5. V_{ss} estimated by 1- and 2-compartment models based on 4 h and 24 h intravenous infusion studies. Each value was normalized to the V_{ss} of IV bolus data, then shown as average fold-difference. The actual mean V_{ss} values for each group were shown in the bracket (L/kg).

	Internal compounds				Commercial compounds					Total (with loperamide)		Total (without loperamide)	
	A	B	C	D	Atenolol	Loperamide	Minoxidil	NFPS	Sulpiride	Average	CV%	Average	CV%
Noncompartmental analysis (NCA)													
IV bolus	1 (2.80)	1 (11.5)	1 (1.42)	1 (3.99)	1 (5.56)	1 (2.72)	1 (6.96)	1 (7.07)	1 (8.25)	1	0	1	0
1-compartment analysis (1-comp)													
1 mg/kg over 4 h	0.99 (2.77)	0.20 (2.29)	1.02 (1.45)	0.50 (2.01)	0.39 (2.14)	0.76 (2.06)	0.35 (2.45)	0.63 (4.43)	0.38 (3.11)	0.58	50.2	0.56	54.4
1 mg/kg over 24 h	1.60 (4.48)	0.45 (5.22)	0.90 (1.29)	0.70 (2.81)	0.61 (3.40)	8.41 (22.9)	0.60 (4.19)	1.04 (7.35)	0.62 (5.13)	1.66	154	0.82	44.9
6 mg/kg over 24 h	1.13 (3.18)	0.17 (1.92)	1.32 (1.87)	0.83 (3.32)	0.68 (3.80)	8.07 (22.0)	0.67 (4.66)	0.72 (5.12)	0.75 (6.19)	1.59	154	0.79	43.6
2-compartment analysis (2-comp)													
1 mg/kg over 4 h	0.99 (2.77)	0.57 (6.51)	1.24 (1.77)	0.34 (1.34)	0.65 (3.63)	0.76 (2.06)	1.27 (8.87)	0.74 (5.23)	0.52 (4.30)	0.79	41.0	0.79	43.6
1 mg/kg over 24 h	1.24 (3.48)	0.83 (9.49)	1.98 (2.81)	0.96 (3.82)	0.61 (3.41)	13.7 (37.3)	1.17 (8.16)	1.09 (7.74)	0.91 (7.50)	2.50	169	1.10	37.1
6 mg/kg over 24 h	1.02 (2.87)	0.60 (6.85)	1.71 (2.42)	1.30 (5.18)	0.83 (4.62)	2.86 (7.79)	0.73 (5.10)	0.73 (5.13)	0.86 (7.07)	1.18	60.7	0.97	37.7

Table 6. $t_{1/2,eff}$ estimated by 1- and 2-compartment models based on 4 h and 24 h intravenous infusion studies. Each value was normalized to the $t_{1/2,eff}$ of IV bolus data, then shown as average fold-difference. The actual mean $t_{1/2,eff}$ values for each group were shown in the bracket (h).

	Internal compounds				Commercial compounds					Total	
	A	B	C	D	Atenolol	Loperamide	Minoxidil	NFPS	Suppiride	Average	CV%
Noncompartmental analysis (NCA)											
IV bolus	1 (1.16)	1 (5.65)	1 (2.25)	1 (15.1)	1 (1.83)	1 (1.43)	1 (0.79)	1 (4.44)	1 (4.18)	1	0
1-compartment analysis (1-comp)											
1 mg/kg over 4 h	1.95 (2.25)	0.16 (0.92)	2.44 (5.48)	0.15 (2.32)	0.23 (0.43)	0.18 (0.26)	0.32 (0.25)	0.26 (1.14)	0.29 (0.34)	0.66	132
1 mg/kg over 24 h	2.71 (3.13)	0.21 (1.21)	1.11 (2.49)	0.56 (8.45)	0.49 (0.89)	2.45 (3.51)	0.60 (0.47)	0.90 (3.99)	0.55 (0.65)	1.06	84.2
6 mg/kg over 24 h	1.27 (1.47)	0.09 (0.50)	1.27 (2.86)	0.93 (14.1)	1.01 (1.84)	2.90 (4.16)	1.01 (0.80)	1.21 (5.38)	1.18 (1.39)	1.21	60.5
2-compartment analysis (2-comp)											
1 mg/kg over 4 h	2.93 (3.39)	0.51 (2.86)	3.00 (6.74)	0.11 (1.72)	0.42 (0.77)	0.18 (0.26)	1.44 (1.13)	0.30 (1.32)	0.40 (0.47)	1.03	113
1 mg/kg over 24 h	4.04 (4.67)	0.81 (4.55)	4.27 (9.61)	1.41 (21.2)	0.49 (0.90)	4.57 (6.56)	1.23 (0.96)	0.97 (4.33)	0.81 (0.95)	2.07	82.1
6 mg/kg over 24 h	2.80 (3.23)	0.32 (1.79)	1.70 (3.83)	4.92 (74.2)	1.25 (2.29)	0.75 (1.08)	1.11 (0.88)	1.21 (5.39)	1.37 (1.61)	1.71	80.6

Figure 1

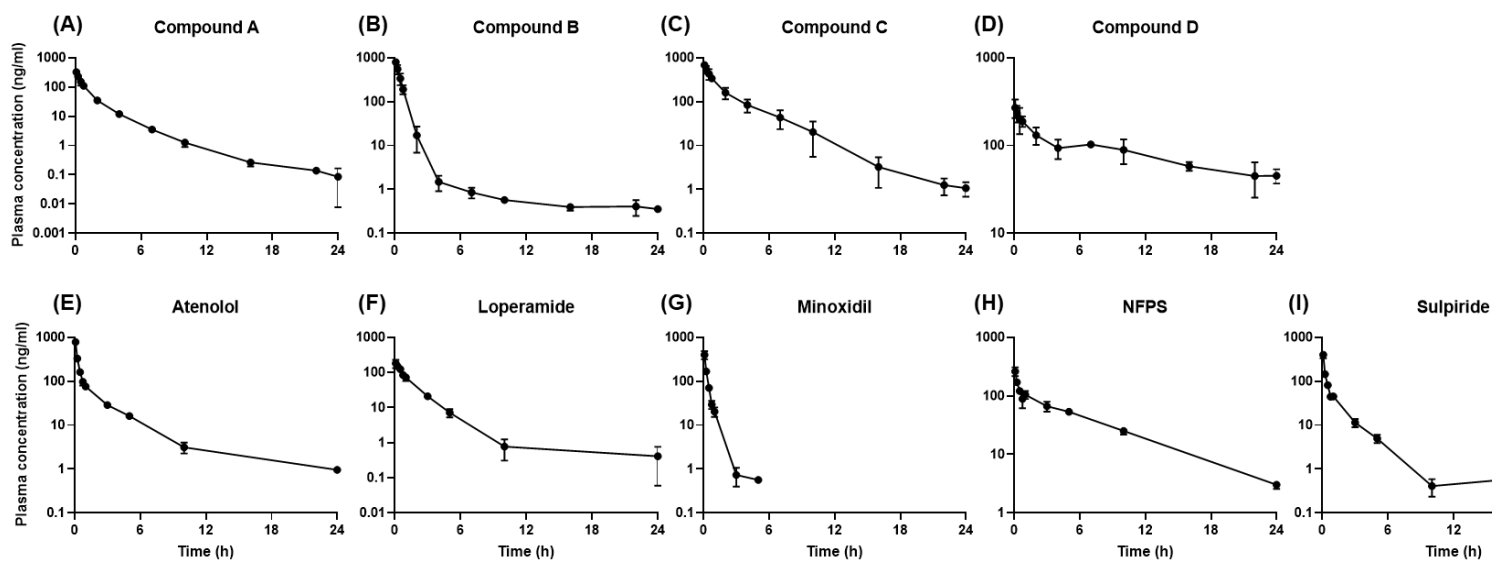


Figure 2

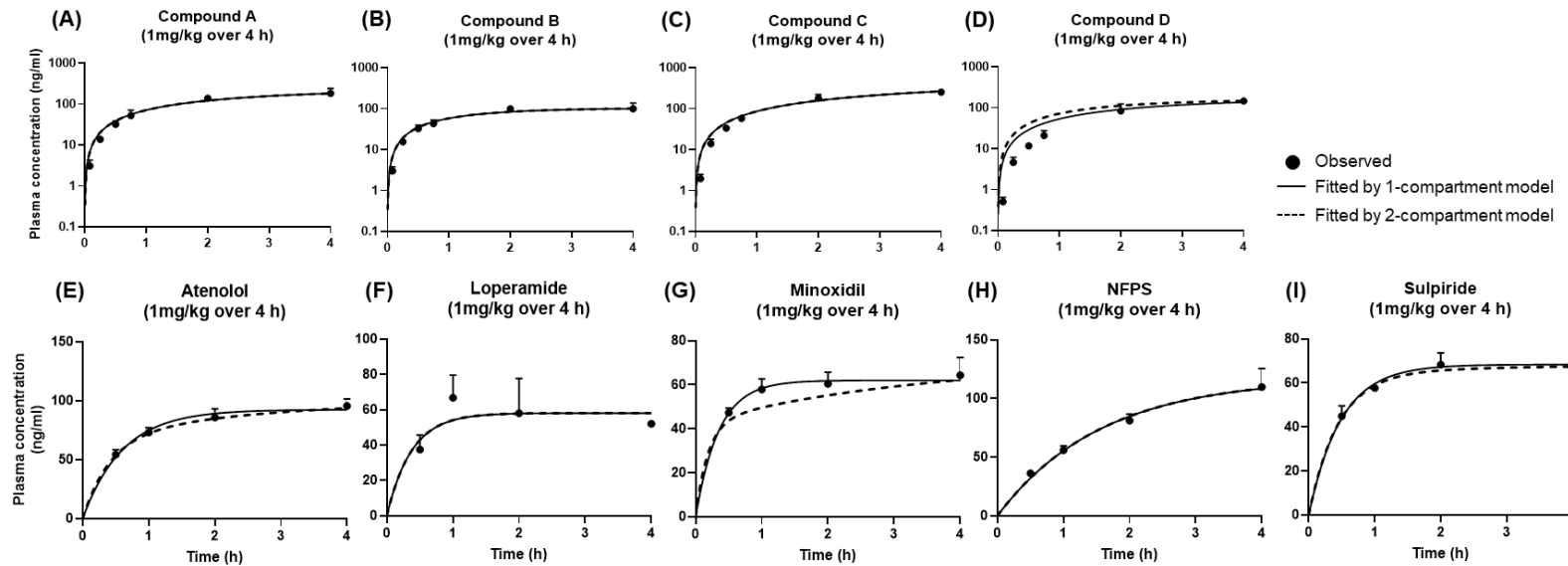


Figure 3

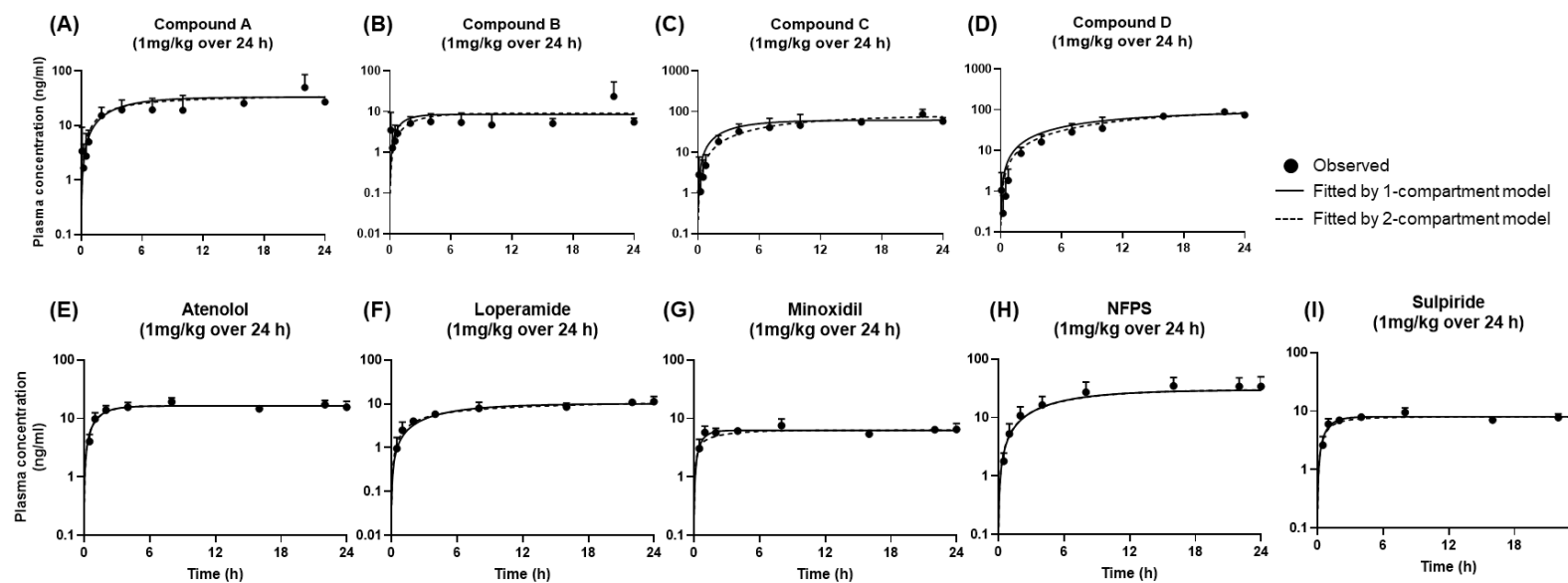


Figure 4

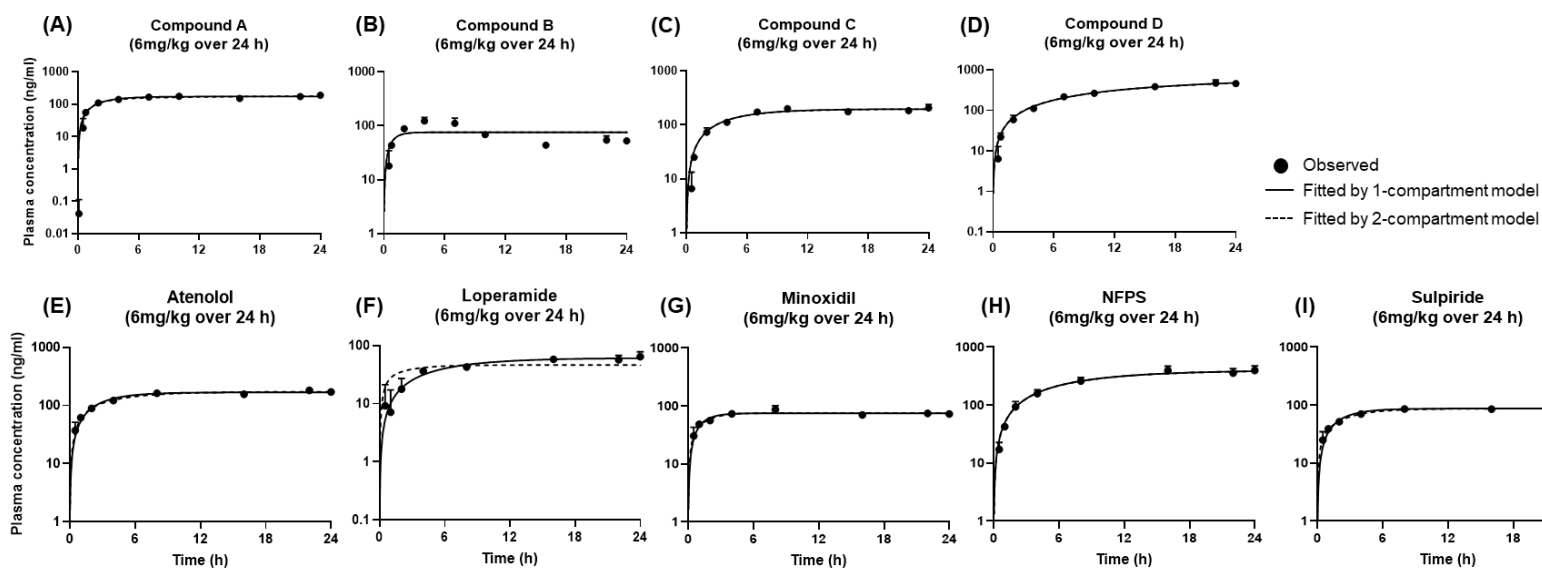


Figure 5

