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First-in-human safety, tolerability, and pharmacokinetics of SY-007, a prolonged action neuroprotective drug for ischemic stroke, in healthy Chinese subjects

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ABSTRACT

Background and purpose: SY-007 is an interfering peptide designed to disrupt the cell death signaling of phosphatase and tensin homolog deleted on chromosome ten (PTEN) nuclear translocation during ischemic stroke. Preclinical studies indicated that rats treated with 1.5 mg/kg SY-007 in the middle cerebral artery occlusion (MACO) model had significantly reduced stroke lesion size even when administered 6 h after the stroke onset. The aim of this study was to evaluate the safety, tolerability, and pharmacokinetics of ascending doses of SY-007 administered intravenously in healthy Chinese subjects.

Methods: A total of 78 healthy Chinese subjects were enrolled in the single ascending dose study (1–60 mg) and received a 15-min intravenous infusion SY-007 or placebo. Plasma concentrations of SY-007 were measured by a validated liquid chromatography-tandem mass spectrometry method. Pharmacokinetic parameters were determined using non-compartmental and compartment analyses. A model based on target-mediated drug disposition was applied. Model evaluation was performed through visual predictive checks and bootstrap analysis.

Results: Across doses of 1–60 mg, SY-007 was well tolerated. All adverse events (AEs) were mild or moderate in intensity, and all resolved without intervention. After infusion, SY-007 plasma concentrations decreased quickly with the mean terminal half-life was shorter than 0.78 h. The area under the concentration-time curve increased with a greater than dose-dependent manner from 1 to 30 mg and resulted in a dose-dependent increased from 30 to 60 mg. The nonlinear phenomenon was well described by a simplified target-mediated drug disposition (TMDD) model.

Conclusions: Intravenous dosing of SY-007 appears to be safe up to a dose of 60 mg. Nonlinear pharmacokinetics was observed across the evaluated doses and TMDD might be the primary reason. The effective dose of SY-007 for neuroprotective effect in patients with ischemic stroke is expected to be 10–30 mg and was recommended for the later multiple ascending dose study of SY-007.

Clinical Trial Registration: Clinicaltrials.gov: NCT04111523.

1. Introduction

Stroke is one of the leading causes of morbidity and mortality worldwide (GBD 2016 Causes of Death Collaborators, 2017; Kim et al., 2020; Phipps and Cronin, 2020; Wu et al., 2019). Over 13 million people will have a stroke each year and around 5.5 million people will die as a result (GBD 2016 Causes of Death Collaborators, 2017; GBD 2016 Stroke Collaborators, 2019; Kim et al., 2020; Phipps and Cronin, 2020; Wu et al., 2019). In China, stroke, with over 2 million new cases annually, is associated with the highest disability-adjusted life-years lost of any disease (Wu et al., 2019). Ischemic stroke, caused by blood clots formed inside the blood vessels with sudden onset, is the most common type of stroke. The goal of ischemic stroke care is to minimize brain injury and maximize patient recovery. The standard pharmacotherapy for ischemic stroke is intravenous thrombolytic therapy with recombinant tissue plasminogen activator (rtPA), which is the only accepted causal

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treatment (Chamorro et al., 2016). However, rtPA is ineffective beyond 4.5 h after stroke onset and, therefore, is only offered to 5% of all patients with stroke (Ge et al., 2020; Prabhakaran et al., 2015). Those disadvantages limit the use of rtPA, and most stroke cases do not have an effective pharmacological treatment option.

N-methyl-D-aspartate glutamate receptors (NMDARs) have important roles in stroke pathology and recovery (Ge et al., 2020). Depending on their subtypes and locations, NMDARs may promote either neuronal survival or death (Ge et al., 2020). However, despite a lot of systematic attempts, NMDAR channel blockers have failed to be translated into clinical stroke treatments (Ge et al., 2020; Wu and Tymianski, 2018; Zhang et al., 2013). Recent research on NMDAR-associated signaling complexes had found that, overactivated GluN2B-containing NMDARs cause phosphatase and tensin homolog deleted on chromosome ten (PTEN) nuclear translocation during cerebral ischemia (Ge et al., 2020; Zhang et al., 2013). PTEN nuclear translocation subsequent resulting in increased neuronal death, and thereby contributing to excitotoxic/ischemic neuronal damage (Ge et al., 2020; Zhang et al., 2013).

In order to disrupt the NMDARs cell death signaling, an interfering peptide SY-007 (NH_2-Lys-Glu-Ile-Val-Ser-Arg-Asn-Lys-Arg-Arg-Tyr-Gln-Glu-Asp-Gly-Arg-Lys-Lys-Arg-Arg-Gln-Arg-Arg-Arg-CH_3COOH) was developed by *Zhang et al.* (Ge et al., 2020; Wu and Tymianski, 2018; Zhang et al., 2013). Preclinical studies have demonstrated that SY-007 inhibiting PTEN nuclear translocation was strongly neuroprotective against *in vitro* NMDA- and *in vivo* ischemia-induced neuronal damage (Ge et al., 2020; Wu and Tymianski, 2018; Zhang et al., 2013). SY-007 does not affect NMDARs, but targets PTEN nuclear translocation, a delayed step in the NMDAR-mediated cell death signaling cascade (Zhang et al., 2013). Rats treated with 1.5 mg/kg SY-007 in the middle cerebral artery occlusion (MACO) model had significantly reduced stroke lesion size even when administered 6 h after the stroke onset, which supports the concept that disrupting the downstream pro-death signaling cascade can provide a wider therapeutic time window than blocking the upstream NMDAR channels (Wu and Tymianski, 2018; Zhang et al., 2013). MRI dosing study revealed that 1.5 mg/kg SY-007 appeared the effective dose for poststroke treatment (unpublished data, being sharing if required, Suzhou Yabao Pharmaceutical R&D Co., Ltd., szyb@yabaoyaoye.com). Due to its efficacy characteristics, the National Medical Products Administration (NMPA) of China approved SY-007 to proceed with clinical trials in 2018. This first-in-human study presents the safety, tolerability, and pharmacokinetics of placebo controlled, ascending doses of SY-007 administered intravenously in healthy Chinese subjects.

2. Methods

2.1. Study design and treatment

This phase I clinical study of SY-007 was a placebo-controlled, double-blind, randomized, single ascending dose study in healthy Chinese subjects. The study started at 1 mg and participants were randomized 2:1 to receive a single dose of SY-007 (n=4) or placebo (n = 2). Then the dose-escalated to 4, 10, 20, 30, 45 and 60 mg, and participants were randomized 5:1 to receive a single dose of SY-007 (n = 10) or placebo (n = 2). In each dose cohort, two participants (one SY-007 and one placebo) were dosed 48 hours before the remaining participants. The remaining participants' dosing was to be staggered if there were safety concerns. SY-007 or placebo was administered in the early clinical research unit (West China Hospital of Sichuan University, Chengdu, China) on day 1 as a 15-min intravenous infusion under the supervision of the investigator. Participants were observed for at least 2 h after completion of the infusion to monitor for infusion-related reactions. Doses were escalated after review of available safety data and each subject only participated in one dose cohort. SY-007 injection, manufactured and packaged by Hangzhou Ausia Biological Technology Company, Ltd. (Hangzhou, China) was diluted in normal saline.

2.2. Participants

Healthy subjects of non-childbearing potential, aged 18–45 years with a body mass index (BMI) ranging from 18 to 26 kg/m^2 and bodyweight > 50 kg for males, > 45 kg for females were included for this study. Within 4 weeks prior to the first administration of the study drug, the subjects were examined if they were deemed healthy based on vital signs (breathing rate, blood pressure, pulse rate, and body temperature), medical history, physical examination, 12-lead ECG, electroencephalogram, Chest X-ray and abdominal ultrasound, brain magnetic resonance imaging, clinical laboratory tests (hematology, biochemistry, coagulation function, thyroid function, and urinalysis), serology (hepatitis B surface antigen, hepatitis C virus antibody, and HIV antigen/antibody) and urine drug screening (methamphetamine, Methylenedioxy methamphetamine, ketamine, morphine, and diamorphine).

Subjects were excluded with any of the following reasons: with a history of nervous system disorder or malignancy; those who reported use of other drugs that might interfere with the study results within 2 weeks of the study drug administration; allergy to any drug or food or those with known hypersensitivity to SY-007 and any components of the formulation; drug or alcohol abuse; female subjects were excluded if they were pregnant or breastfeeding; subjects were also excluded if they participated in a clinical study within 3 months prior to screening.

2.3. Pharmacokinetic assessments

A series of venous blood samples were collected for determinations of SY-007 at pre-dose (within 0.5 h prior to dosing) and at 5, 10, 15, 20, 30, 45 min, and 1, 1.5, 2, 3, 4, 5, 6 h after the beginning of the intravenous infusion. Plasma concentration of SY-007 was determined using a validated liquid chromatography-tandem mass spectrometry method. In brief, the validated method was linearity over the concentration range of 2.10 to 1050 ng/mL using a plasma sample volume of 0.1 mL. The intra-batch precision and accuracy ranged from 1.4 to 8.9% and 85.4 to 105.8%, respectively. The inter-batch precision and accuracy ranged from 6.7 to 8.9% and 93.7 to 97.6%, respectively. SY-007 in plasma was stable after five cycles of freeze (−30, −80°C) and thaw, at room temperature for 24 h and in the refrigerator at −30°C for 79 days and at −80°C for 474 days (Wang et al., 2021).

PK parameters were calculated by non-compartmental analyses using Phoenix WinNonlin (version 8.3; Certara, Princeton, NJ, USA)). Maximum observed plasma concentration (C_{max}) and time to C_{max} (T_{max}) were obtained directly from the concentration-time curves of the observed values. The area under the plasma concentration-time curves from the time of dosing to the time of the last observation ($AUC_{0\text{-}t}$) and to infinity ($AUC_{0\text{-}\infty}$) were estimated by the linear trapezoidal rule and $AUC_{0\text{-}t}$ + the AUC extrapolated from the last data point to infinity (AUC_{ex}), where AUC_{ex} was estimated by dividing the concentration at the last point by the elimination rate constant (λ_z). The terminal elimination half-life ($t_{1/2}$) was calculated by using the terminal elimination rate constant (λ_z) and the equation $t_{1/2} = \ln_2/\lambda_z$, where λ_z was estimated by log-linear regression after semilogarithmic transformation of the SY-007 data, using the last three data points of the terminal linear phase of the concentration-time curve of SY-007. Clearance (CL) was calculated with the formula of dose/$AUC_{0\text{-}\infty}$. Volume of distribution (V_d/F) were calculated as $V_d = CL/\lambda_z$. Mean residence time (MRT) was calculated from the ratio of $AUMC_{0\text{-}t}$ /$AUC_{0\text{-}t}$, where $AUMC_{0\text{-}t}$ was the calculation of the area under the first-moment curve (plasma concentration × time vs. time).

Additionally, compartment pharmacokinetic analysis was performed on NONMEM 7.2 (GloboMax, Hanover, MD, USA). The first-order conditional estimation with interaction method (FOCEI) and ADVAN 6 (general nonlinear kinetics) subroutine were used to obtain the typical population parameters, random inter-individual variability, random inter-occasion variability on portion parameters, and residual variability between observed and individually predicted plasma concentrations.

Covariates were tested for statistical significance, clinical relevance, and scientific interest. The covariates included in the analysis were sex, age, weight, body mass index (BMI) and dose. A stepwise forward inclusion and backward elimination process was used for covariate searching (Wählby et al., 2002). Visual predictive checks (VPC) were performed to evaluate the predictive performance of the final pharmacokinetic model. Nonparametric bootstrap analysis was also performed. Monte Carlo simulations of SY-007 drug concentration-time curve were performed for 1000 individuals based on the final models.

2.4. Safety and tolerability assessment

Adverse events (AEs) and serious adverse event (SAEs) were monitored throughout the study. Safety assessments comprised examination of vital signs, 12-lead ECG, clinical laboratory, and physical examination at specified times after administration. Vital signs were performed at admission and at 0.25, 0.5, 1, 6, 24, 48 h, and day 7 after administration. 12-leads ECG was performed at admission, within 0.25 h prior to dosing, at 0.25, 0.5, 1.5, 6, 24, 48 h, and day 7 after administration. Clinical laboratory examination was performed at admission and on day 3 and day 7. Additionally, ECG monitoring was performed from the beginning of testing until 2 h after drug administration. All treated subjects underwent inpatient observation for 3 days after infusion, with follow-up assessments at day 7 after administration of SY-007 for each dose cohort.

2.5. Statistical analysis

Descriptive statistics were used to summarize the demographic data, pharmacokinetic parameters, and safety data. The Power model was used to investigate dose proportionality of SY-007 ranging from 1 to 60 mg with the following equation: Ln (AUC_{0-t}, $AUC_{0-\infty}$ or C_{max}) = $\beta_0 + \beta_1 \times$ Ln (dose). The estimate of the slope of the regression line (β_1) and the corresponding 95% CI were calculated. All statistical analyses were performed by using the SAS system, version 9.4 (SAS Institute Inc., Cary, NC, USA), a P-value less than 0.05 is statistically significant.

3. Results

3.1. Participants

A total of 78 eligible healthy Chinese subjects (48 males and 30 females) were enrolled, 64 subjects received SY-007, and 14 subjects received placebo. The safety analysis included all the randomized subjects in the studies. PK analysis included all subjects who received SY-007 in the studies. The demographic characteristics of the subjects are summarized in Table 1. In brief, subjects had a mean age of 24.8 years (range, 19–38 years), a mean body weight of 60.2 kg (range, 45.1–82.7 kg), and a mean BMI of 21.7 kg/m^2 (range, 18.1–26.0 kg/m^2).

3.2. Safety and tolerability

Single intravenous doses of SY-007 up to 60 mg were well tolerated, and no significant safety concerns emerged during the study. In total, 45 AEs were reported from 28 of the 78 subjects in the study (Table 2). 14 of these were related to SY-007 in 9 subjects. The most frequently reported AEs included urine leukocyte positive, rash, and pruritus. These were urine leukocyte positive in 6 subjects who received SY-007 doses of 4, 10, 45, 60 mg, and placebo (2 subjects). 4 subjects in the doses of 45, and 60 mg, and placebo (2 subjects) experienced rash and 4 subjects in the doses of 1, 45, and 60 mg, and placebo experienced pruritus. All AEs were mild or moderate in intensity, and all resolved without intervention. There was no SAE reported, no subjects were withdrawn from the study due to AEs. Vital signs, including breathing rate, blood pressure, pulse rate, and body temperature, laboratory tests (hematology, biochemistry, coagulation function, thyroid function) and urinalysis were within normal limits and remained largely unchanged from baseline in all participants.

3.3. Pharmacokinetic non-compartmental analyses

Mean SY-007 plasma concentration–time profiles following intravenous administration to healthy Chinese subjects are displayed in Fig. 1A andB. Mean pharmacokinetic parameters of SY-007 are listed in Table 3. In brief, SY-007 concentrations generally increased during infusion and then decreased with time. The mean SY-007 plasma concentration was measurable 1 h after 1 mg dosing, and up to 6 h as the dose-escalated to 60 mg. The plasma kinetic profile of SY-007 exhibited a steep disposition phase, with plasma concentrations quickly falling below the limit of quantitation (2 ng/ml) and a switch from fast clearance at a low dose to a longer $t_{1/2}$ at a higher dose, which suggested the presence of nonlinear pharmacokinetics. The nonlinear characteristics were confirmed by the dose-normalized concentration-time profiles (Fig. 1C). In order to visualize the nonlinear phenomenon clearly, $AUC_{0-\infty}$ values were normalized by dividing the values by the SY-007 dose (Fig. 2A). The estimated regression coefficients and 95% confidence intervals for C_{max}, AUC_{0-t}, and $AUC_{0-\infty}$ determined by the power model were 1.0402 (0.9948–1.0856), 1.2660 (1.2212–1.3108), and 1.2470 (1.2030–1.2909), respectively. Although the 95% confidence interval for C_{max} includes 1, the 95% confidence intervals for AUC_{0-t} and $AUC_{0-\infty}$ did not include 1, which verified the greater than dose-dependent manner. Besides, mean pharmacokinetic parameters showed that $AUC_{0-\infty}$ and C_{max} increased with increasing dose in a greater than dose-

Table 1
Baseline demographic characteristics of study participants (N = 78). Values are given as mean (SD) unless otherwise indicated.

Characteristics	1 mg (n=4)	4 mg (n=10)	10 mg (n=10)	20 mg (n=10)	30 mg (n=10)	45 mg (n=10)	60 mg (n=10)	Placebo (n=14)
Age, year								
Mean (SD)	25.5 (4.51)	23.4 (3.37)	25.7 (5.66)	25.2 (6.51)	25.1 (3.41)	25.0 (4.45)	23.5 (2.42)	25.2 (2.78)
Median (range)	24.0 (22–32)	23.0 (20–32)	23.5 (21–37)	23.0 (19–38)	24.0 (21–31)	23.5 (21–32)	24.0 (19–28)	25.0 (21–32)
Sex, n (%)								
Male	2 (50)	6 (60)	5 (50)	7 (70)	5 (50)	8 (80)	8 (80)	7 (50)
Female	2 (50)	4 (40)	5 (50)	3 (30)	5 (50)	2 (20)	2 (20)	7 (50)
Race, n (%)								
Han	4 (100)	8 (80)	9 (90)	9 (90)	10 (100)	10 (100)	10 (100)	13 (93.6)
Other	0	2 (20)	1 (10)	1 (10)	0 (0)	0 (0)	0 (0)	1 (6.4)
Weight, kg								
Mean (SD)	55.5 (7.21)	64.3 (12.7)	61.2 (9.20)	59.7 (11.1)	55.4 (7.52)	58.8 (7.92)	65.0 (7.79)	59.0 (7.02)
Median (range)	52.7 (50.4–66.1)	63.1 (47.3–81.8)	60.8 (47.4–74.8)	57.4 (45.1–76.2)	52.8 (48.4–72.4)	58.8 (48.3–72.3)	65.0 (50.7–82.7)	57.7 (49.3–71.4)
BMI, kg/m^2								
Mean (SD)	20.8 (1.55)	21.6 (2.30)	22.2 (1.91)	21.6 (3.23)	21.2 (2.39)	21.0 (2.16)	22.7 (1.80)	21.8 (1.73)
Median (range)	21.0 (19.0–22.3)	22.4 (18.4–25.0)	22.15 (19.7–25.4)	20.0 (18.3–26.0)	20.5 (18.4–26.0)	20.2 (18.1–24.7)	22.2 (20.1–25.8)	21.7 (19.7–25.7)

Abbreviations: SD, standard deviation; BMI, body mass index.

Table 2
Events of adverse events occurring in at least two subjects after a 15 min intravenous infusion of SY-007 to healthy Chinese volunteers.

Adverse event	1 mg (n=4)	4 mg (n=10)	10 mg (n=10)	20 mg (n=10)	30 mg (n=10)	45 mg (n=10)	60 mg (n=10)	Placebo (n=14)
Any AEs, n (%)	1 (25.0)	2 (20.0)	2 (20.0)	0	4 (40.0)	4 (40.0)	7 (70.0)	8 (57.1)
Urine leukocyte Positive	0	1 (10.0)	1 (10.0)	0	0	1 (10.0)	1 (10.0)	2 (14.3)
Rash	0	0	0	0	0	1 (10.0)	1 (10.0)	2 (14.3)
Pruritus	1 (25.0)	0	0	0	0	1 (10.0)	1 (10.0)	1 (7.1)
Diarrhea	0	0	0	0	0	0	1 (10.0)	2 (14.3)
Sinus bradycardia	0	0	1 (10.0)	0	0	0	0	1 (7.1)
Dry mouth	0	0	0	0	0	0	2 (20.0)	0
Neutrophil count increased	0	0	0	0	1 (10.0)	0	0	1 (7.1)
Upper respiratory infection	0	1 (10.0)	0	0	0	0	0	1 (7.1)

Abbreviations: AE, adverse events.

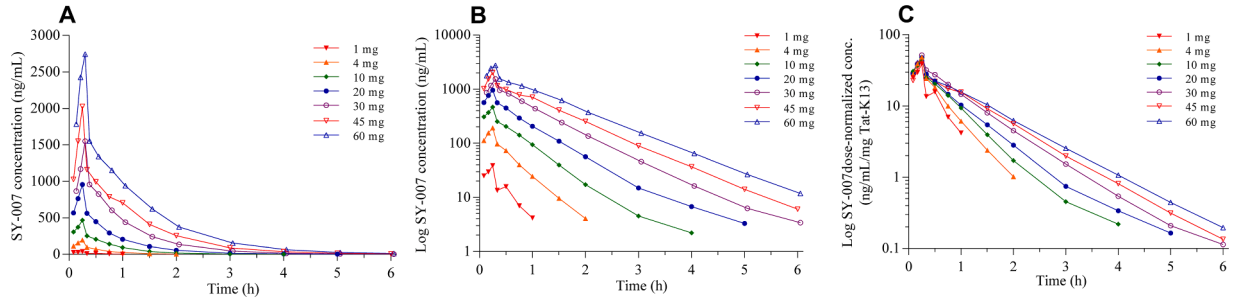

Fig. 1. Mean plasma concentration–time curves after administration of SY-007 injection in healthy Chinese subjects. A. Mean plasma concentration–time curves following single dose administration; B. Log Mean plasma concentration–time curves following single dose administration; C. The log dose-normalized SY-007 concentration-time following single dose administration.

Table 3
Pharmacokinetic parameters after a 15 min intravenous infusion of SY-007 to healthy Chinese volunteers.

Parameters	1 mg (n=4)	4 mg (n=10)	10 mg (n=10)	20 mg (n=10)	30 mg (n=10)	45 mg (n=10)	60 mg (n=10)
T_{max} (h)	0.25 (0.08–0.33)	0.25 (0.17–0.25)	0.25 (0.25–0.25)	0.25 (0.17–0.25)	0.25 (0.17–0.25)	0.25 (0.25–0.27)	0.25 (0.17–0.25)
C_{max} (ng/mL)	35.8 ± 13.7	195 ± 45.4	468 ± 106	984 ± 106	1560 ± 301	2030 ±430	2750 ± 540
AUC_{0-t} (h·ng/mL)	13.3 ± 5.00	91.5 ± 22.6	267 ± 57.2	625 ± 109	1170 ± 258	1700 ±318	2490 ± 564
$AUC_{0-\infty}$ (h·ng/mL)	14.9 ± 5.59	94.0 ± 22.8	271 ± 56.4	628 ± 109	1180 ± 258	1710 ±319	2510 ± 571
AUC_{ex} (%)	10.6 ± 3.31	2.68 ± 1.35	1.32 ± 0.90	0.55 ± 0.14	0.34 ± 0.12	0.38 ± 0.16	0.52 ± 0.18
$t_{1/2}$ (h)	0.25 ± 0.02	0.36 ± 0.04	0.45 ± 0.05	0.55 ± 0.07	0.70 ± 0.08	0.73 ± 0.05	0.78 ± 0.06
V_d (L)	28.6 ± 14.8	23.5 ± 7.04	24.5 ± 4.81	25.6 ± 2.75	26.9 ± 6.98	28.7 ± 5.45	28.2 ± 6.46
CL (L/h)	78.6 ± 41.0	45.3 ± 12.9	38.7 ± 9.56	32.9 ± 7.16	26.7 ± 6.37	27.1 ± 4.96	25.3 ± 6.99
MRT (h)	0.38 ± 0.09	0.41 ± 0.04	0.54 ± 0.06	0.66 ± 0.08	0.80 ± 0.05	0.96 ± 0.15	1.01 ± 0.08

Abbreviations: T_{max}, the time to maximum observed plasma concentration; C_{max}, the maximum observed plasma concentration; AUC_{0-t}, the area under the plasma concentration-time curves from the time of dosing to the time of the last observation; $AUC_{0-\infty}$, the area under the plasma concentration-time curves from the time of dosing to infinity; AUC_{ex}, the AUC extrapolated from the last data point to infinity; $t_{1/2}$, terminal elimination half-life; CL, clearance. $V_{d,}$ volume of distribution; MRT, mean residence time.

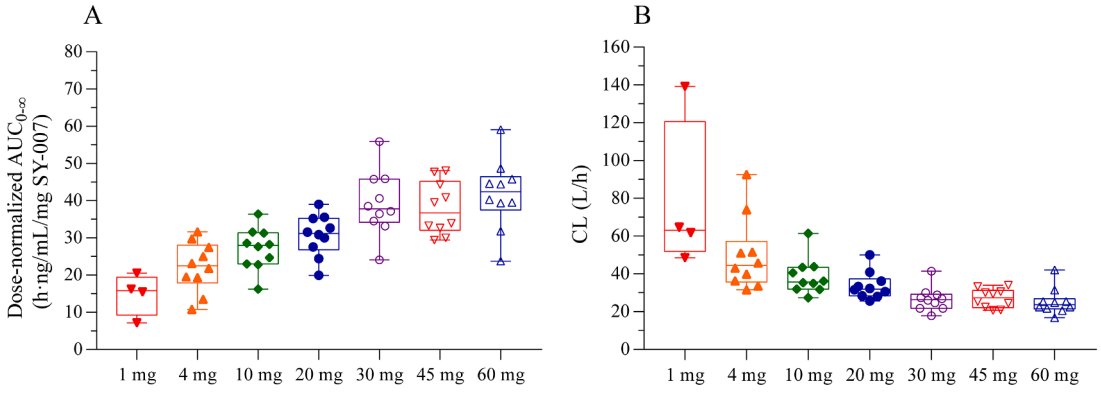

Fig. 2. The pharmacokinetic parameters of SY-007 injection in healthy Chinese subjects. A. the dose-normalized $AUC_{0-\infty}$ of SY-007 in healthy Chinese subjects across the evaluated dose range; B. the clearance of SY-007 in healthy Chinese subjects across the evaluated dose range.

dependent manner from 1 to 30 mg. However, $AUC_{0-\infty}$ and C_{max} increased nearly dose-proportionally from 30 to 60 mg after intravenous administration in healthy Chinese subjects. The same phenomenon could also be observed on CL (Fig. 2B), suggesting that the systemic elimination of SY-007 was dose-dependent.

3.4. Pharmacokinetic compartmental analyses

The compartmental analysis dataset was comprised of 770 SY-007 plasma concentrations from 64 subjects. A total of 126 samples (14.1 %) were reported as being below LLOQ, and were handled utilizing the Beal M3 method (Beal, 2001). A two-compartment model with first-order absorption and target-mediated drug disposition was shown to be the most appropriate base model for SY-007. The structure of the pharmacokinetic model is represented schematically in Fig. 3., which was defined by the following differential equations:

$$\frac{dA_c}{dt} = In(t) - \frac{CL + CL_d}{V_c} \times A_c + \frac{CL_d}{V_p} \times A_p - \frac{(R_{max} - DR) \times K_2 \times \frac{A_c}{V_c}}{K_d + \frac{A_c}{V_c}} + K_{off} \times DR \; Ac(0) = 0$$

$$\frac{dA_p}{dt} = \frac{CL_d}{V_c} \times A_c - \frac{CL_d}{V_p} \times A_p Ap(0) = 0$$

$$\frac{dDR}{dt} = \frac{(R_{max} - DR) \times K_2 \times \frac{A_c}{V_c}}{K_d + \frac{A_c}{V_c}} - \left(K_{off} + K_{int}\right) \times DR DR(0) = 0$$

where A_c and A_p represent the amount of SY-007 in central and peripheral compartment, respectively. System parameters include apparent amount of binding partner (R_{max}), the amount of SY-007 binding complex (DR), the first order rate constant for conversion of the low affinity complex to the high affinity complex (K_2), the equilibrium dissociation constant (K_d), the first order rate constant for dissociation of intact hetrombopag from binding complex (K_{off}), the rate constant for target-mediated disposition (K_{int}), clearance of the central compartment (CL), volume of distribution for the central compartment (V_c), volume of distribution for the peripheral compartment (V_p), clearance between central compartment and peripheral compartment (CL_d). The total SY-007 plasma concentration was defined by the following differential equations:

$$C_{tol} = \frac{A_c}{V_c} + \frac{DR}{V_c}$$

A proportional residual error model was finally selected after comparing with other residual error models.

The parameter estimates are reported in Table 4. No remarkable covariate on pharmacokinetic parameters was found out. The goodness-of-fit plots (Fig. 4) and VPC (Fig. 5) for the compartmental model indicated that the model described the data adequately. Of 500 bootstrap runs completed, 12 failed to minimize successfully. The parameter estimates from bootstrap were similar to the NONMEM estimates from the final pharmacokinetic model, supporting the stability of the pharmacokinetic model and the good precision of parameter estimates. The pharmacokinetic steady state curve of SY-007 in healthy Chinese subjects after a 15 min intravenous infusion of 10 mg, 20 mg, 30 mg and 60 mg every six hours (Q6h) were performed by Monte Carlo simulations (Fig. 6). The resulted indicated that there was no obvious accumulation at the steady-state following repeat dosing.

4. Discussion

This placebo-controlled, double-blind, randomized, single ascending dose study was the first study that evaluated the safety, tolerability, and pharmacokinetics of SY-007 in humans. The drug in a single-intravenous dose of up to 60 mg was well tolerated in healthy Chinese subjects. The overall incidence of AEs was not dose-dependent and was qualitatively similar to those seen with placebo and were generally mild, transient, and self-limited. Single doses of SY-007 did not cause serious adverse events or discontinuations. No clinically significant treatment-related effects were apparent with respect to vital signs, laboratory tests, electrocardiograms, electroencephalograms for all SY-007 dose cohorts included in this study.

The FIH starting dose and dose range of SY-007 in healthy volunteers were determined based on the no observable adverse effect level (NOAEL) and the effective dose in animal species. One of the key steps for interspecies extrapolation is to predict human equivalent dosage from animal dosage. In our study, the human equivalent doses (HEDs) was extrapolated to 50 mg and 100 mg based on the NOAELs of SY-007 on rat and dog were 5 mg/kg and 3 mg/kg, respectively. In order to provide a margin of safety for protection of human subjects receiving the initial clinical dose, a safety factor of 50 was then be applied to generate the maximum recommended starting dose (MRSD), namely, 1 mg, in humans. Besides, the FIH dose levels of SY-007 in healthy volunteers

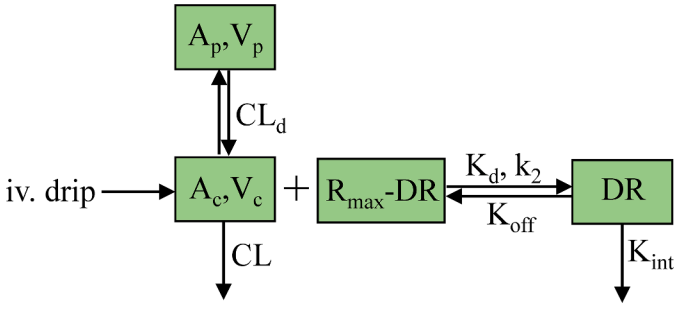

Fig. 3. The structure of the pharmacokinetic model. CL, clearance of the central compartment; CL_d, clearance between central compartment and peripheral compartment; V_c, volume of distribution for the central compartment; V_p/F, volume of distribution for the peripheral compartment; R_{max}, apparent amount of binding partner; K_d, equilibrium dissociation constant; K_{off}, dissociation of intact hetrombopag from binding complex; K_{int}, rate constant for target-mediated disposition; k_2, rate constant for conversion of the low affinity complex to the high affinity complex.

Table 4
Parameter estimates of the Population PK Model.

Parameters	Final Model		Bootstrap Estimates	
	Point Estimate	RSE%[a]	Mean	95% CI[b]
CL (L/h)	24.7	5.00	5.01	22.3–27.1
CL_d (L/h)	142	11.1	11.1	111–173
V_c (L)	2.62	31.7	31.6	0.997–4.24
V_p (L)	21.1	6.20	6.18	18.5–23.7
R_{max} (μM)	2.59	12.4	12.2	1.97–3.21
K_d (h^{-1})	3.26	31.6	31.6	1.24–5.28
K_{off} (h^{-1})	0.283	24.0	23.9	0.150–0.416
K_{int} (h^{-1})	2.66	37.9	38.1	0.674–4.65
k_2 (h^{-1})	9.5	25.4	25.3	4.79–14.2
ω_{CL} (%)	33.3	22.5 (0.2)	22.5	18.6–48.0
ω_{Vc} (%)	76.5	31.0 (17.7)	31.2	29.7–123
ω_{Vp} (%)	16.9	28.4 (13.2)	28.1	7.59–26.2
σ_{prop} (%)	14.2	2.40 (10.9)	2.40	13.5–14.9

Abbreviations: CL, clearance of the central compartment; CL_d, clearance between central compartment and peripheral compartment; V_c, volume of distribution for the central compartment; V_p/F, volume of distribution for the peripheral compartment; R_{max}, apparent amount of binding partner; K_d, equilibrium dissociation constant; K_{off}, dissociation of intact hetrombopag from binding complex; K_{int}, rate constant for target-mediated disposition; k_2, rate constant for conversion of the low affinity complex to the high affinity complex.
[a] percent relative standard error of the estimate (shrinkage%).
[b] 95% confidence interval on the parameter.

covered the anticipated therapeutic dose range. In this study, allometric scaling (AS) and in vitro in vivo extrapolation (IVIVE) was used to predict human anticipated therapeutic dose (Lombardo et al., 2013; Sharma and McNeill, 2009). The therapeutic dose range of SY-007 in rats was 1.5-4.5 mg/kg and the interspecies extrapolation result indicated that the therapeutic dose range in human was 10-30 mg, and thus, the maximum dose was be pre-defined as 60 mg in this FIH single ascending dose study.

Except for 1 mg and 45 mg cohorts, the T_{max} of SY-007 were observed at 0.17–0.25 h across the evaluated doses. The C_{max} of one participant in 1 mg cohort occurred at 5 min (0.08 h) after the infusion started with unknown reason. In general, drugs administered by constant intravenous infusion show a zero-order input process and the T_{max} generally occurred at the end of the infusion. However, if the elimination process is rapid, the C_{max} of intravenous administration would be influenced by various factors, such as participant's body position changing, transfusion infiltration and performance of infusion pump. Actually, the elimination of SY-007 was very rapid, with the mean elimination $t_{1/2}$ lower than 0.78 h. SY-007 showed nonlinear pharmacokinetics with a greater than dose-proportional increase across the evaluated doses, which also been observed in preclinical animal studies (unpublished data, being sharing if required, Suzhou Yabao Pharmaceutical R&D Co., Ltd., szyb@yabaoyaoye.com). The nonlinear elimination phenomenon mainly occurred at lower SY-007 doses (1–30 mg). The dose-normalized plasma concentration–time profiles indicated that the elimination phase was going up as the dose increases with C_{max} non-significantly increased. This behavior could be explained by the theory of target-mediated drug disposition (Mager and Jusko, 2001). Exhibiting target-mediated drug disposition imply that SY-007 could bind to a high-affinity–low-capacity target, which is a prevalence phenomenon in large-molecule compounds (Mager and Jusko, 2001). After SY-007 binds to its target, a significant fraction of SY-007-target complex molecules will undergo subsequent target-mediated internalization, endocytosis, and degradation, which increased the speed of SY-007 elimination. In the current study, the clearance of SY-007 was faster at a low dose (78.6 ± 41.0 L/h) owing to that, at a low dose, binding to target has a significant impact on clearance. As the dose increases, the target is saturated owing to its low capacity, and the fraction of dose that binds to the target is minimal compared with the total dose (An, 2020; Mager and

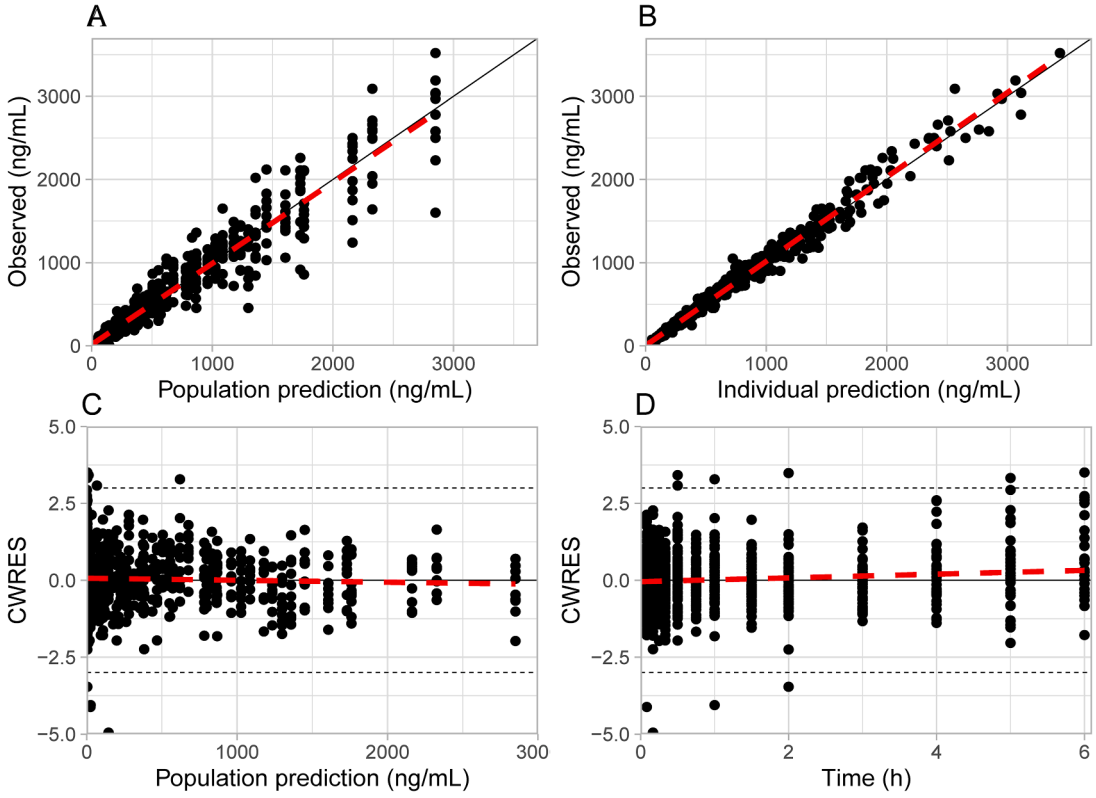

Fig. 4. Goodness of fit plots for the final PK model. A. population predicted versus the observed concentration. B. individual predicted versus the observed concentration. C. conditional weighted residual versus the predicted concentration. D. Conditional weighted residual versus time. Dashed red line represents local regression smoothing line through the data. dotted black line represents the ideal conditional weighted residual.

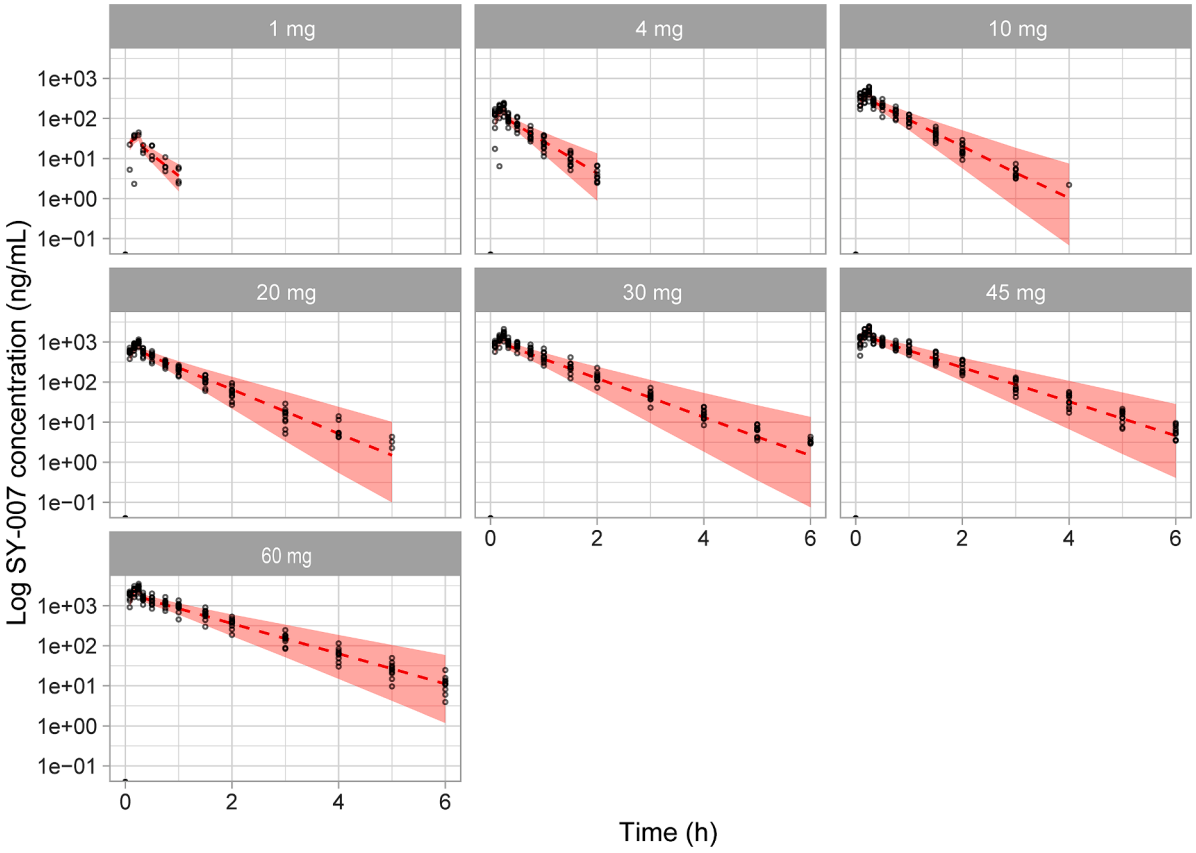

Fig. 5. Visual predictive check for the PK model. The shadow shows the 90% prediction interval and the dotted line shows the median of the simulated concentration-time profiles. The observed hetrombopag plasma concentrations are displayed as dots.

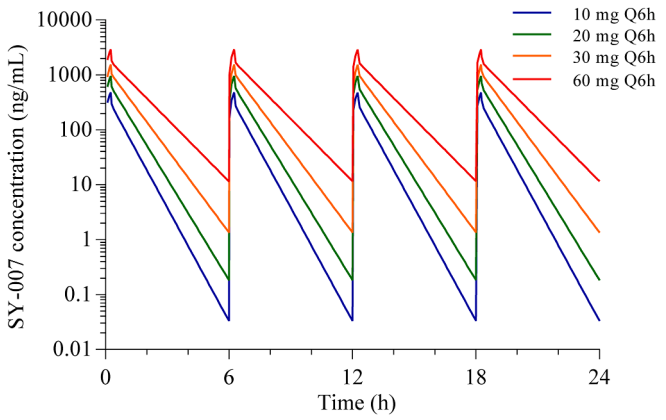

Fig. 6. The simulation concentration–time curves after a 15 min intravenous infusion of SY-007 10 mg, 20 mg, 30 mg and 60 mg every six hours (Q6h) to healthy volunteers.

Jusko, 2001). As a result, the kinetics of the system is approximation dose-proportional at high doses, which could be supported by the exposure to SY-007 increased in a dose-proportional manner in the dose range of 30–60 mg. In order to better describe the nonlinear phenomenon, a simplified target-mediated drug disposition model as described previously was employed (Landersdorfer et al., 2012). The result indicated that target-mediated drug disposition explains the dose-dependent elimination $t_{1/2}$ and the more than dose-proportional increase in $AUC_{0-\infty}$ appropriately.

The prediction of the dose-response relationship in patients with ischemic stroke is crucial for later research decisions. However, the pharmacodynamic response was not investigated in this study owing to that all participants were healthy subjects. SY-007 was designed to disrupt the cell death signaling of PTEN nuclear translocation in patients with ischemic stroke (Ge et al., 2020; Wang et al., 2021; Wu and Tymianski, 2018; Zhang et al., 2013). Before clinical studies begin, the pharmacodynamic behavior of SY-007 in patients with ischemic stroke could only rely on interspecies extrapolation. One key assumption in our interspecies pharmacodynamic extrapolation of SY-007 was that same exposure produces same response between humans and animals (Liu et al., 2016). The effective dose significantly reduced stroke lesion size in rat MACO model was 1.5 mg/kg with the mean $AUC_{0-\infty}$ was 276–899 h·ng/mL (unpublished data, being sharing if required, Suzhou Yabao Pharmaceutical R&D Co., Ltd., szyb@yabaoyaoye.com). The equivalent exposure level that might be the expected effective dose in patients with ischemic stroke was 10–30 mg ($AUC_{0-\infty}$ was 271–1180 h·ng/mL). Of course, the extrapolation of preclinical animal studies to predict the likely pharmacokinetic properties of drugs in humans are often poor predictors and needs to be verified by clinical studies in later stage (Bracken, 2009). Another key factor for later clinical studies is the dosing intervals of SY-007 in patients with ischemic stroke. Due to the target mediated drug disposition that leads to nonlinear pharmacokinetics, the determination of the dosing intervals of SY-007 was guided with the pharmacokinetic modeling approach. The result indicated that take a SY-007 dose every six hours (Q6h) will be maintained adequate exposure and anticipated therapeutic index with no obvious accumulation at the steady-state. Thus, the dosage regimen of 10–30 mg every six hours is recommended for the later multiple ascending dose study of SY-007 in healthy subjects.

5. Conclusions

Single intravenous doses of 1 mg to 60 mg of SY-007 were safe and well-tolerated by the healthy subjects in this first-in-human study. Nonlinear pharmacokinetics was observed and described by the target-mediated drug disposition model. Peak concentrations (C_{max}) increased in a dose-proportional manner. The area under the concentration-time curve increased with a greater than dose-dependent manner from 1 to 30 mg and increased with a dose-dependent manner from 30 to 60 mg. Based on the model simulation and preclinical pharmacodynamic data, the effective dose of SY-007 for neuroprotective effect in patients with ischemic stroke is expected to be 10–30 mg, which was recommended for the later multiple ascending dose study of SY-007.

Ethics approval

The clinical study protocol was approved by the Ethics Committee at the West China Hospital of Sichuan University (Chengdu, China). Clinical procedures were conducted in the Early Clinical Research Ward of Clinical Trial Center of West China Hospital of Sichuan University. The study (Clinicaltrials.gov: NCT04111523) were conducted in accordance with the Declaration of Helsinki and the Guidelines for Good Clinical Practice.

Conflict of Interest

The authors have no conflict of interest to declare.

CRediT authorship contribution statement

Zhenlei Wang: Conceptualization, Methodology, Formal analysis, Funding acquisition, Writing – original draft. **Ying Jin:** Software, Validation, Formal analysis, Visualization, Writing – original draft. **Qi Shen:** Data curation, Writing – review & editing. **Lang Zhuo:** Resources, Project administration. **Runhan Liu:** Investigation, Writing – review & editing. **Xiangjie Di:** Data curation, Writing – original draft. **Lisha Xiang:** Investigation, Funding acquisition. **Guanyu Zhang:** Resources. **Ying Wang:** Investigation. **Yongsheng Wang:** Supervision. **Li Zheng:** Supervision, Conceptualization, Methodology, Investigation, Funding acquisition, Writing – review & editing.

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References

An, G., 2020. Concept of pharmacologic target-mediated drug disposition in large-molecule and small-molecule compounds. J. Clin. Pharmacol. 60 (2), 149–163.
Beal, S.L., 2001. Ways to fit a PK model with some data below the quantification limit. J. Pharmacokinet. Pharmacodyn. 28, 481–504.
Bracken, M.B., 2009. Why animal studies are often poor predictors of human reactions to exposure. J. R. Soc. Med. 102, 120–122.
Chamorro, Á, Dirnagl, U, Urra, X, Planas, AM., 2016. Neuroprotection in acute stroke: targeting excitotoxicity, oxidative and nitrosative stress, and inflammation. Lancet Neurol. 15 (8), 869–881.
GBD 2016 Causes of Death Collaborators, 2017. Global, regional, and national age-sex specific mortality for 264 causes of death, 1980-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet 390 (10100), 1151–1210.
GBD 2016 Stroke Collaborators, 2019. Global, regional, and national burden of stroke, 1990-2016: a systematic analysis for the Global Burden of Disease Study 2016. Lancet Neurol. 18 (5), 439–458.
Ge, Y, Chen, W, Axerio-Cilies, P, Wang, YT., 2020. NMDARs in Cell Survival and Death: Implications in Stroke Pathogenesis and Treatment. Trends Mol. Med. 26 (6), 533–551.
Kim, J, Thayabaranathan, T, Donnan, GA, et al., 2020. Global stroke statistics 2019. Int. J. Stroke, 1747493020909545.
Landersdorfer, C.B., He, Y.L., Jusko, W.J., 2012. Mechanism-based population pharmacokinetic modelling in diabetes: vildagliptin as a tight binding inhibitor and substrate of dipeptidyl peptidase IV. Br. J. Clin. Pharmacol. 73, 391–401.
Liu, D., Ma, X., Liu, Y., Zhou, H., Shi, C., Wu, F., Jiang, J., Hu, P., 2016. Quantitative prediction of human pharmacokinetics and pharmacodynamics of imigliptin, a novel DPP-4 inhibitor, using allometric scaling, IVIVE and PK/PD modeling methods. Eur. J. Pharm. Sci. 89, 73–82.
Lombardo, F., Waters, N.J., Argikar, U.A., Dennehy, M.K., Zhan, J., Gunduz, M., Harriman, S.P., Berellini, G., Liric Rajlic, I., Obach, R.S., 2013. Comprehensive assessment of human pharmacokinetic prediction based on in vivo animal pharmacokinetic data, part 2: clearance. J. Clin. Pharmacol. 53, 178–191.
Mager, DE, Jusko, WJ., 2001. General pharmacokinetic model for drugs exhibiting target-mediated drug disposition. J. Pharmacokinet. Pharmacodyn. 28 (6), 507–532.
Phipps, MS, Cronin, CA., 2020. Management of acute ischemic stroke. BMJ 368, l6983.
Prabhakaran, S, Ruff, I, Bernstein, RA., 2015. Acute stroke intervention: a systematic review. JAMA 313 (14), 1451–1462.
Sharma, V., McNeill, J.H., 2009. To scale or not to scale: the principles of dose extrapolation. Br. J. Pharmacol. 157, 907–921.
Wang, Z, Jia, S, Zhang, Q, Wang, Y, Huang, B, Zheng, L., 2021. LC-MS/MS assay for the determination of Tat-K13, a novel interfering peptide for the treatment of ischemic stroke, in human plasma and its application to a pharmacokinetics study. Biomed. Chromatogr. e5095.
Wu, QJ, Tymianski, M., 2018. Targeting NMDA receptors in stroke: new hope in neuroprotection. Mol. Brain 11 (1), 15.
Wu, S, Wu, B, Liu, M, et al., 2019. Stroke in China: advances and challenges in epidemiology, prevention, and management. Lancet Neurol. 18 (4), 394–405.
Wählby, U., Jonsson, E.N., Karlsson, M.O., 2002. Comparison of stepwise covariate model building strategies in population pharmacokinetic-pharmacodynamic analysis. AAPS PharmSci. 4, E27.
Zhang, S, Taghibiglou, C, Girling, K, et al., 2013. Critical role of increased PTEN nuclear translocation in excitotoxic and ischemic neuronal injuries. J. Neurosci. 33 (18), 7997–8008.