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Virtual population pharmacokinetic using physiologically based pharmacokinetic model for evaluating bioequivalence of oral lacidipine formulations in dogs

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ABSTRACT

The aim of the present study was to investigate virtual population pharmacokinetic using physiologically based pharmacokinetic (PBPK) model for evaluating bioequivalence of oral lacidipine formulations in dogs. The dissolution behaviors of three lacidipine formulations including one commercial product and two self-made amorphous solid dispersions (ASDs) capsules were determined in 0.07% Tween 80 media. A randomized 3-period crossover design in 6 healthy beagle dogs after oral administration of the three formulations at a single dose of 4 mg was conducted. The PBPK modeling was utilized for the virtual bioequivalence study. *In vitro* dissolution experiment showed that the dissolution behaviors of lacidipine amorphous solid dispersions (ASDs) capsules, which was respectively prepared by HPMC-E5 or Soluplus, as polymer displayed similar curves compared with the reference formulation in 0.07% Tween 80 media. *In vivo* pharmacokinetics experiments showed that three formulations had comparable maximum plasma drug concentration (C_{max}), and the time (T_{max}) to reach C_{max} of lacidipine tablet, which was prepared by Soluplus, as polymer was slower than other two formulations in consistency with the *in vitro* dissolution rate. The 90% confidence interval (CI) for the C_{max}, $AUC_{0-24\,h}$ and $AUC_{0-\infty}$ of the ratio of the test drug to the reference

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drug exceeded the acceptable bioequivalence (BE) limits (0.80–1.25). However, the 90% CI of the $AUC_{0-24\,h}$, $AUC_{0-\infty}$ and C_{max} of the ratio of test to reference drug were within the BE limit, calculated using PBPK modeling when the virtual subjects reached 24 dogs. The results all demonstrated that virtual bioequivalence study can overcome the inequivalence caused by inter-subject variability of the 6 beagle dogs involved in *in vivo* experiments.

1.   Introduction

During the generic drug approval process, the approvement for marketing requires that the generic drug demonstrate both pharmaceutical equivalence and BE between the generic product and its corresponding reference formulation (R) [1]. BE studies are an integral component of the new drug development process and played an important role in the approval and marketing of generic drug products [2]. Generally, *in vivo* bioequivalence studies are conducted using a single-dose, randomized, two-period crossover design [3]. The plasma concentration profile of the generic drug product is compared to that of reference, and two products are considered to be bioequivalent if they show the same rate and extent of absorption [2]. According to the criteria developed by the U.S. Food and Drug Administration and generally applied by other regulatory agencies, the test and reference formulations were considered to be bioequivalent when the 90% confidence interval of the geometric mean ratios of C_{max}, AUC_{0-t} and $AUC_{0-\infty}$ between the reference and the test formulations was within the range of 80.00%–125.00% [2].

However, the results of a BE study can be impacted by factors including sample size and variability other than true differences in C_{max} and/or AUC between the test formulations and the reference formulation [4]. Enhancing the experimental subject size or utility accurate simulation model will be desirable. Usually, the researchers conducted the BE study with a large number of subject, which would result in great cost and high labor. Physiologically based pharmacokinetic (PBPK) model is such a feasible mechanistic tool to predict the PK of drug products for virtual bioequivalent study.

PBPK model is a mathematical model that integrates physicochemical properties of drug substances, formulation properties of drug products and physiological parameters of animals to predict absorption, distribution, metabolism and excretion of compounds *in vivo* [5–7]. A large number of methodologies including allometric scaling [8–11] and physiologically based pharmacokinetic (PBPK) model [12,13] have been established for PK prediction. PBPK model is a more comprehensive mechanistic tool to predict the optimized PK parameters, and predict plasma and tissue concentration–time profiles of drug products than conventional model [14,15]. Additionally, use of PBPK models enables the prediction of human plasma concentration time profiles with minimal (and in some cases no) animal data [5]. Currently, population pharmacokinetics simulation by using the PBPK model to obtain the PK of drug products from large subjects has not been discussed and explored extensively.

In this work, the aim of the present study was to investigate virtual population pharmacokinetic using PBPK model for evaluating bioequivalence of oral lacidipine formulations in dogs. The dissolution behaviors of three lacidipine formulations were determined in 0.07% Tween 80 media. A randomized 3-period crossover design in 6 healthy beagle dogs after oral administration of three formulations at a single dose of 4 mg was conducted. The physiologically based pharmacokinetic (PBPK) model was utilized for virtual BE studies. In summary, the virtual BE studies will enhance the accuracy of the experiment of bioequivalence with minimal animal data and reduce repeated *in vivo* experiment.

2.   Materials and methods

2.1.   Materials

Lacidipine was purchased from Kangya of NingXia Pharmaceuticals Co. Ltd. (Ningxia, China). Soluplus and HPMC-E5 were gifts from BASF Co., Ltd. (Shanghai, China). Capsule shell was purchased from Suzhou Capsule Co. Ltd. (Suzhou, China). Sodium hydroxide pellets, sodium chloride, sodium dihydrogen phosphate monohydrate, ethanol, dichloromethane and Tween 80 were analytical grade and purchased from Tianjin Bodi Chemical Holding Co. Ltd. (Tianjin, China). Formic acid was purchased from Dikma Scientific Inc. (Beijing, China). Methanol, acetonitrile and tert-butyl methyl ether were of chromatographic grade and purchased from Thermo Fisher Scientific Inc. (USA). Heparin sodium was purchased from Tianjin Biochemistry Pharmaceuticals Co. Ltd. (Tianjin, China). Nimodipine reference substance was purchased from the National Institutes for Food and Drug Control (Beijing, China). Deionized-distilled water was used throughout the study.

2.2.   Formulations

Three formulations of lacidipine were determined in this study. The reference formulation of lacidipine (R) was LACIPIL® 4 mg tablet (GlaxoSmithKline, England). Two test formulations of lacidipine were capsules that respectively contain two kinds of lacidipine amorphous solid dispersion. The amorphous solid dispersions (ASDs) were prepared via solvent evaporation technique. Two ASDs were prepared by the following step: Drug/polymer weight ratios of lacidipine and polymers were 1:12.5. One polymer was HPMCE5 (T_1), while another was Soluplus (T_2). Then lacidipine and polymers were dissolved in a 1:1 *v/v* mixture of dichloromethane and ethanol, and organic

Table 1 – The compositions of the blank fasted state intestinal fluid.

Composition	
NaH_2PO_4	1.719 g
NaCl	3.093 g
NaOH	0.174 g
pH	6.5
Deionized water	500 ml

solvents were removed by rotary evaporation. The samples were dried under vacuum for about 12 h at room temperature. The dried samples formed a uniform and free flowing powder by passing through the 80 mesh sieve.

2.3. Media preparation

The compositions of the blank fasted state intestinal fluid (Blank FaSSIF) [16] were summarized in Table 1. The 0.07% Tween 80 media was prepared by adding 0.7 g Tween 80 to 1 l blank FaSSIF.

2.4. Dissolution tests

Dissolution experiments were conducted according to the USP Apparatus 2 (Paddle) setup (ZRS-8G; Tianda Tianfa Technology Co., Ltd, Tianjin, China). Each vessel was filled with 500 ml of 0.07% Tween 80 media, which was degassed before the dissolution tests proceeded and equilibrated at 37 °C. The rotation speed was set at 50 rpm. 5 ml of samples were withdrawn at 5, 10, 20, 30, 45 and 60 min, the same amount of fresh medium were replaced at predetermined time intervals. Then withdrawn samples filtered through 0.45 µm filters. Dissolution for each formulation was carried out in triplicate ($n = 3$). Withdrawn samples were measured by HPLC method.

2.5. In vivo pharmacokinetic study

2.5.1. Animals
The in vivo pharmacokinetic study was conducted with ethical permission, which was permitted by Ethical Committee in China and was processed in accordance with the Guide for the Care and Use of Laboratory Animals. The pharmacokinetic study of three lacidipine formulations was performed on six beagle dogs weighing 12–16 kg. These dogs were divided into 3 groups randomly, and the study was carried out in a crossover experimental design with a washout period of one week. The dogs were fasted for about 12 h prior to experiments and were given water freely. The preparations were administered orally at a single dose of 4 mg. 2.0 ml of blood samples were taken into a heparinized blood collection tube via a detaining needle at pre-dose, and 0.25, 0.50, 0.75, 1.0, 1.5, 2.0, 3.0, 4.0, 6.0, 8.0, 12.0 and 24.0 h post-dose. The dogs were provided with a standard lunch 4 h after dosing. The plasma was obtained by centrifuging the blood samples at 3500 rpm for 10 min, and stored at −20 °C.

2.5.2. Bioanalytical method
The lacidipine plasma concentrations were determined by a validated ultra performance liquid chromatography-dual mass spectrometry (UPLC-MS/MS) method after liquid–liquid extraction by tert-butyl methyl ether with nimodipine as internal standard. The chromatographic separations were acquired on an ACQUITY UPLC™ system (Waters Corp., Milford, MA, USA) and Thermo C18 column (50 mm × 2.1 mm, 2.6 µm; Thermo Fisher Scientific, USA) with a mobile phase composed of acetonitrile and water containing 0.1% formic acid (83:17; v/v) at a flow rate of 0.2 ml/min. The quantitation was performed by Waters Tandem Quadrupole (TQ) Detector (Waters). The mass spectrometer was operated with electrospray ionization (ESI) source in positive ionization mode and the compounds were analyzed by multiple reaction monitoring (MRM) of the transitions of m/z 473.47→354.28 for lacidipine and m/z 419.25→343.18 for nimodipine, respectively.

2.5.3. Data analysis
Non-compartmental model analysis was conducted to calculate the main pharmacokinetic parameters. The maximum plasma drug concentration (C_{max}) and the time (T_{max}) to reach C_{max} of the three formulations were directly gained from the experimental data. The half-life ($t_{1/2}$) was calculated using DAS 2.1.1 software. The area under the plasma concentration–time curve up to the last measured time point (AUC_{0-t}) was determined using the trapezoidal rule. The relative bioavailability (F) was the ratio between the test formulations (T_1 and T_2) and the reference formulation (R), and:

$$F = \frac{AUC_{0\text{-}t\ test}}{AUC_{0\text{-}t\ reference}} \times 100\%$$

it was expressed as following equation:

$AUC_{0-24\ h}$, $AUC_{0-\infty}$ and C_{max} make logarithmic transformation. ANOVA of $lnAUC_{0-24\ h}$, $lnAUC_{0-\infty}$ and lnC_{max} were calculated for three formulations, and the threshold for differences to be considered significant was set at $P \leq 0.05$. Two one-sided t tests were used to evaluate whether the 90% confidence intervals (CIs) of the geometric mean ratios (test: reference) for C_{max}, $AUC_{0-24\ h}$ and $AUC_{0-\infty}$ were within the range of 80.00%–125.00% (using log transformed data).

2.6. Virtual bioequivalence study

2.6.1. PBPK model in dog based on the literature data
Physiologically based pharmacokinetic models are composed of a series of differential equations and have been implemented in a number of commercial software packages. In this study, all the in vivo PK simulations were conducted in the PBPK model commercial software GastroPlus (Version 8.6.0003; Simulations Plus, Inc., Lancaster, CA, USA). Input parameters in the PBPK model can be categorized into three classes: physicochemical properties (such as solubility, pKa, LogP and permeability), formulation properties (such as drug particle size and dissolution profiles), and pharmacokinetic parameters (such as clearance, volume of distribution, and the disposition model). The main drug-specific input parameters of log P, blood to plasma partitioning, mean particle radius and Particle density are in silico predicted by ADME Predictor. The Diffusion coefficient, volume of distribution at steady state (V_{ss}) and elimination half-time in dog are calculated by GastroPlus

Table 2 – PBPK model of lacidipine based on the literature data.

Parameters	Value
MW	455.55
LogP	5.51[a]
Dog permeability (P_{eff}, cm/sec × 10^4)	2.5[a]
Mean particle radius (μm)	25[b]
Mean precipitation time (sec)	900[b]
Particle density (g/ml)	1.2[b]
Diffusion coefficient (cm^2/sec × 10^5)	0.6139[c]
Solubility (mg/ml)	5×10^{-5} in blank FaSSIF[d]
Unbound percent in plasma (F_{up}, %)	5[d]
Clearance (CL, l/h)	9.6[d]
Volume of distribution (V_{ss}, l)	159.45[c]
Elimination half-time ($T_{1/2}$, h)	11.5[c]
Simulation time (h)	24

[a] Predicted by ADME Predictor.
[b] Default GastroPlus™.
[c] Calculated by GastroPlus™.
[d] Literature value[17–19].

Fig. 1 – Dissolution profiles of three lacidipine formulations in 0.07% Tween 80 media. Data are presented as the mean ± SD (n = 3). (The reference formulation of lacidipine (R), lacidipine/HPMC-E5 capsules (T$_1$) and lacidipine/Soluplus capsules (T$_2$)).

equations. The lacidipine solubility in media and unbound percent in plasma (F_{up}) in dogs is based from literatures [17,18], respectively. The clearance (CL) is an important drug-specific parameter required for PBPK modeling. Lacidipine is mainly eliminated by hepatic metabolism, and renal clearance can be negligible. The rate of hepatic metabolism in dogs is based from a publication [19]. All these parameters for the simulation are summarized in Table 2.

In our previous study, a PKPB model was successfully built and validated to simulate PK parameters for the lacidipine in dogs [17]. In this study, population pharmacokinetics simulation of the three lacidipine formulations was carried out on the successfully built PBPK model.

2.6.2. PK simulation for three lacidipine formulations in dogs
The *in vivo* PK simulations were conducted in the PBPK model commercial software GastroPlus (Version 8.6.0003, Simulations Plus, Inc., Lancaster, CA, USA). After all parameters (Table 2) were imported into the successfully built PKPB model of lacidipine, the dissolution profiles of the three formulations in 0.07% Tween 80 solution were loaded to measure the effect of formulations and dissolution conditions on the PK profiles. The imported physiology properties (such as blood flow, volume of tissues, etc.) were changed within 3 times range to conduct the virtual population pharmacokinetic simulation. The change of the imported physiology properties parameters stimulated different dogs and resulted in different values of C_{max} and AUC. The dissolution profiles of the three formulations in 0.07% Tween 80 solution that loaded to the model could get the difference PK profiles of R, T$_1$ and T$_2$. After importing 24 groups of physiology properties parameters, the PBPK model commercial software GastroPlus outputted 24 groups of C_{max} and AUC for each formulation.

3. Results and discussion

3.1. In vitro *dissolution study*

Biorelevant media have been successfully adapted to simulate human small intestinal fluids and have been proven
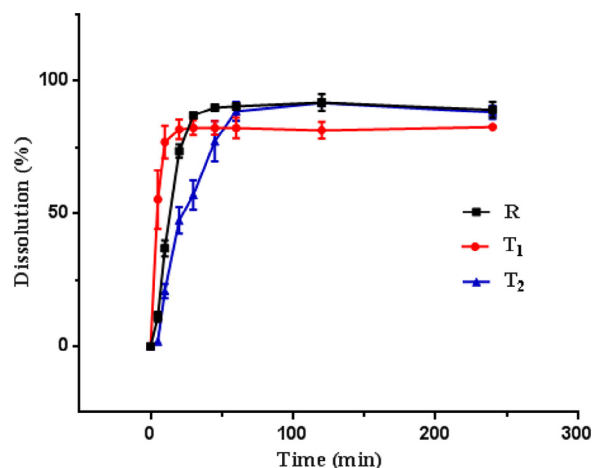
valuable in establishing IVIVC for poorly soluble drugs [20,21]. In our previous work, we have investigated that 0.07% Tween 80 solution is a simple alternative medium to the simulated fasted state intestinal fluid instead of the biorelevant media [22]. In this study, the 0.07% Tween 80 media was chosen as the dissolution media.

The dissolution profiles of three different lacidipine formulations in 0.07% Tween 80 media are shown in Fig. 1. The dissolution profile of three lacidipine formulations at 50 rpm showed the 100% dissolution less than 1 h trailed by plateau phase up to 4 h. The dissolution of capsules containing Soluplus ASD of lacidipine was nearly complete (>85%) in media after 1 h, although the dissolution rate was slightly slower than other two formulations. It concluded that the self-made Soluplus ASD and HPMC-E5 ASD of lacidipine exhibited similar dissolution behavior compared with that of LACIPIL® in 0.07% Tween 80 media. According to their *in vitro* dissolution results, the three different lacidipine formulations in 0.07% Tween 80 media may have perfect bioequivalence *in vivo* performances.

3.2. In vivo *pharmacokinetic study*

3.2.1. Pharmacokinetic parameters study
The relevant pharmacokinetics parameters are listed in Table 3, and the mean concentration–time profiles of three formulations of lacidipine are shown in Fig. 2. The maximum plasma drug concentration (C_{max}) values of the reference and the two test formulations were 24.45 ± 6.53, 28.80 ± 11.89 and 26.647 ± 4.44 respectively. This indicated that the three lacidipine formulations have comparable values of C_{max}. The mean T_{max} values of the reference and the two test formulations were 1.13 ± 0.70, 1.29 ± 0.64 and 1.79 ± 1.36 respectively. Regarding the absorption rate, reference and HPMC-E5 ASD of lacidipine (T$_1$) had shorter time to reach the C_{max} than Soluplus ASD of lacidipine (T$_2$). The Soluplus ASD of lacidipine (T$_2$) had larger T_{max} values than the other two formulations, indicating that the Soluplus ASD of lacidipine (T$_2$) exhibited a slow *in vivo*
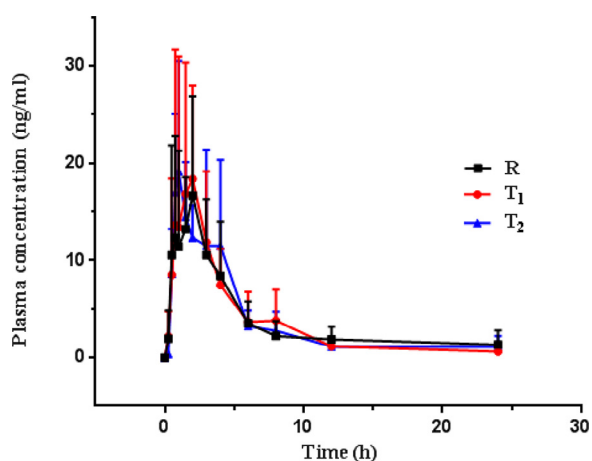
Table 3 – Pharmacokinetic parameters for lacidipine formulations including R, T_1 and T_2 (Data were shown as mean ± SD, $n = 6$)

PK parameters	R	T_1	T_2
C_{max} (ng/ml)	24.45 ± 6.53	28.80 ± 11.89	26.647 ± 4.44
T_{max} (h)	1.13 ± 0.70	1.29 ± 0.64	1.79 ± 1.36
$t_{1/2}$ (h)	8.39 ± 4.60	7.10 ± 6.73	5.20 ± 6.16
AUC_{0-24} (ng·h/ml)	89.16 ± 34.63	87.73 ± 35.72	89.29 ± 31.87
$AUC_{0-\infty}$ (ng·h/ml)	109.37 ± 58.12	94.48 ± 40.38	96.97 ± 40.73
F (%)	100	112.2 ± 57.8	110.6 ± 51.6

dissolution rate, and the result was similar to the *in vitro* dissolution rate of T_2 in 0.07% Tween 80 media. The relative bioavailability of HPMC-E5 ASD of lacidipine (T_1) compared with the reference was 112.2% ± 57.8%, and the Soluplus ASD of

Fig. 2 – Plasma concentration–time profiles for lacidipine commercial tablets and lacidipine ASDs after oral administration of 4 mg in beagle dogs (data are shown as mean ± SD, $n = 6$).

lacidipine (T_2) compared with the reference was 110.6% ± 51.6%. Both of two test formulations slightly enhance the bioavailability.

3.2.2. Bioequivalent analysis

If the 90% CIs of the geometric mean ratios (test: reference) for C_{max}, $AUC_{0-24 h}$ and $AUC_{0-\infty}$ was within the range of 80.00%–125.00%, the test formulations and the reference were considered bioequivalent [23]. The two one-sided *t* tests and 90% CIs results of AUC and C_{max} were summarized in Tables 4 and 5. Wilcoxon signed test of T_{max} was summarized in Table 6.

As shown in Table 4, the 90% CIs of the geometric mean ratios (test: reference) for $AUC_{0-24 h}$ and $AUC_{0-\infty}$ was not within the range of 80.00%–125.00% by using AUC as the evaluation parameters. Additionally, Table 5 showed that 90% CIs of the geometric mean ratios (test: reference) for C_{max} was not within the range of 80.00%–125.00% by using C_{max} as the evaluation parameters. Wilcoxon signed test results (Table 6) of T_{max} between the reference and the two test formulations indicated no significant difference ($P > 0.05$) [3].

In *in vitro* dissolution experiments, the results indicated that both the reference and the two test formulations could totally dissolve within 1 h, so the three formulations had comparable dissolution. The dissolution rate of Soluplus ASD in 0.07% Tween 80 media was slightly slower than the other two for-

Table 4 – Two one side *t*-test results of main parameters between T_1 and R.

Parameters	t_1	t_2	$t_{1-0.05(4)}$	90% Confidence
AUC_{0-24}	0.777	0.999	$t_1 < t_{1-0.05(4)}$, $t_2 < t_{1-0.05(4)}$	49.8%~156.1%
$AUC_{0-\infty}$	0.315	1.138	$t_1 < t_{1-0.05(4)}$, $t_2 < t_{1-0.05(4)}$	56.8%~132.8%
C_{max}	2.203	1.088	$t_1 > t_{1-0.05(4)}$, $t_2 < t_{1-0.05(4)}$	75.4%~169.0%

Table 5 – Two one side *t*-test results of main parameters between T_2 and R.

Parameters	t_1	t_2	$t_{1-0.05(4)}$	90% Confidence
AUC_{0-24}	0.950	0.826	$t_1 < t_{1-0.05(4)}$, $t_2 < t_{1-0.05(4)}$	63.6%~162.1%
$AUC_{0-\infty}$	0.459	0.993	$t_1 < t_{1-0.05(4)}$, $t_2 < t_{1-0.05(4)}$	52.0%~163.1%
C_{max}	2.127	1.165	$t_1 > t_{1-0.05(4)}$, $t_2 < t_{1-0.05(4)}$	74.2%~166.3%

Table 6 – Wilcoxon signed test results of T_{max} between R and T_1.

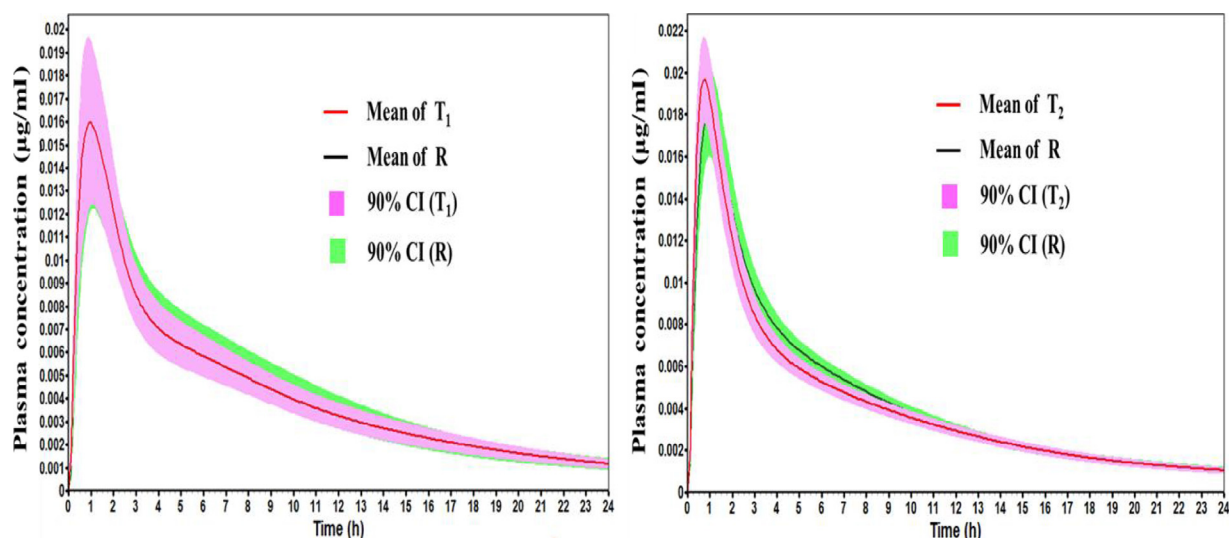|	R	T_1	T_2	P	Conclusion
Mean ± SD	1.13 ± 0.70	1.29 ± 0.64	1.79 ± 1.36	>0 .05	Meeting
Max–Min	2.00–0.50	2.00–0.50	4.00–0.75		the criteria
Median	0.88	1.25	1		

Fig. 3 – Virtual bioequivalent study of reference tablet (R) and test formulations (T₁ and T₂) in 24 beagles.

mulations, and the dissolution rate of Soluplus ASD *in vivo* exhibited the same results. However, bioequivalent analysis indicated that the reference and the two test formulations were inequivalent in the beagle dog *in vivo* experiment. The sample size and variability influenced the outcome of bioequivalent study other than true differences in C_{max} and/or AUC between reference and test formulations. So it might be related with the less experimental animal sample and inter-subject variability [24].

3.3.   *Virtual bioequivalent analysis*

To investigate whether the formulations would be bioequivalent with a large group of experimental animal subjects, the PBPK modeling built in above section was used to simulate pharmacokinetics for the three formulations [17]. A virtual bioequivalent study was conducted to analyze that pharmacokinetics of formulations was influenced by the inter-subject physiological variability.

Fig. 3 showed that the mean plasma concentration–time curves of the reference tablet (R) and the test formulations (T₁ and T₂) all fall in the 90% CIs. The 90% CIs of the geometric mean ratios (T₂: R) for bioequivalent analysis obtained from virtual crossover bioequivalent study was narrower than the 90% CIs of the geometric mean ratios (T₁: R) for bioequivalent analysis. Meanwhile, the simulated mean plasma concentration–time curve of the reference tablet (R) was covered by the 90% CIs of T₁. As shown in Table 7, the 90% CIs of the

geometric mean ratios (test: reference) for C_{max} and AUC was within the requested range as well as meeting the conditions of bioequivalence when the experimental animal sample reached 24. The results here further demonstrated that the reference and the two test formulations were inequivalent when 6 beagle dogs involved in the experiment had inter-subject physiological variability. However, three formulations were equivalent when studying with a large sample.

The 90% CIs of the geometric mean ratios (test: reference) for bioequivalent analysis obtained from virtual crossover bioequivalent study were narrower than the results getting from experimental data *in vivo* study. During the simulation experiment, the same subject had identical physiological and pharmacokinetics parameters when they were administered with the reference and the two test formulations, whereas the physiological and pharmacokinetics parameters of the same subject would be influenced in different occasions, different times or after giving various formulations in an actual experiment [25].

In our previous study, the PBPK model has been validated to simulate PK parameters for the lacidipine self-made micronized tablet and commercial tablets in dogs compared with the observed. In this study, the PBPK model was used as an auxiliary approach to determine bioequivalent study and help reduce the number of animals used. With the ability to predict the input parameters for improving PBPK models, PK can be accurately predicted using *in silico* inputs only [5].

4.   Conclusions

The *in vitro* dissolution profiles of three formulations between the reference and the tests exhibited similar dissolution behavior in 0.07% Tween 80 media. With the less experimental animal sample and inter-subject variability, bioequivalent analysis indicated that the reference and the two test formulations were inequivalent in the beagle dog *in vivo* experiment. Through the population pharmacokinetics simulation using PBPK model

Table 7 – Summary of Virtual BE Studies for lacidipine formulations.

Parameters	T₁ *vs.* R		T₂ *vs.* R	
	Mean ratio	90% CI	Mean	90% CI
C_{max}	1.01	81.6% ~ 124%	1.10	98.4% ~ 123.2%
AUC_{0-24}	1.00	85.8% ~ 115.7%	0.94	87.5% ~ 102.0%
$AUC_{0-\infty}$	1.00	85.4% ~ 116.2%	0.95	87.8% ~ 101.7%

and virtual bioequivalent analysis with 24 virtual subjects, the 90% CIs of the geometric mean ratios (test: reference) for C_{max} and AUC were within the requested BE range as well as meeting the conditions of bioequivalence. Thus, the virtual BE studies will play a potentially useful tool for drug development field.

Acknowledgments

REFERENCES

[1] Jiang W, Makhlouf F, Schuirmann DJ, et al. A bioequivalence approach for generic narrow therapeutic index drugs: evaluation of the reference-scaled approach and variability comparison criterion. AAPS J 2015;17:891–901.
[2] U.S. Food and Drug Administration Center for Drug Evaluation and Research. Guidance for industry: Bioavailability and bioequivalence studies for orally administered drug products – general considerations. Rockville: Food and Drug Administration, <http://www.fda.gov/downloads/Drugs/Guidances/ucm070124.pdf>; 2014 [accessed 15.12.14].
[3] Baek IH, Lee BY, Kang W, et al. Comparison of average, scaled average, and population bioequivalence methods for assessment of highly variable drugs: an experience with doxifluridine in beagle dogs. Eur J Pharm Sci 2010;39:175–180.
[4] Patterson SD, Zariffa NM, Montague TH, et al. Non-traditional study designs to demonstrate average bioequivalence for highly variable drug products. Eur J Clin Pharmacol 2001;57:663–670.
[5] Jones HM, Gardner IB, Watson KJ. Modelling and PBPK simulation in drug discovery. AAPS J 2009;11:155–166.
[6] Benjamin B, Barman TK, Chaira T, et al. Integration of physicochemical and pharmacokinetic parameters in lead optimization: a physiological pharmacokinetic model based approach. Curr Drug Discov Technol 2010;7:143–153.
[7] Jiang W, Kim S, Zhang X, et al. The role of predictive biopharmaceutical modeling and simulation in drug development and regulatory evaluation. Int J Pharm 2011;418:151–160.
[8] Boxenbaum H. Interspecies scaling, allometry, physiological time, and the ground plan of pharmacokinetics. J Pharmacokinet Biopharm 1982;10:201–227.
[9] Mahmood I, Balian JD. Interspecies scaling: predicting pharmacokinetic parameters of antiepileptic drugs in humans from animals with special emphasis on clearance. J Pharm Sci 1996;85:411–414.
[10] Obach RS, Baxter JG, Liston TE, et al. The prediction of human pharmacokinetic parameters from preclinical and in vitro metabolism data. J Pharmacol Exp Ther 1997;283:46–58.
[11] Lave T, Coassolo P, Reigner B. Prediction of hepatic metabolic clearance based on interspecies allometric scaling techniques and in vitro-in vivo correlations. Clin Pharmacokinet 1999;36:211–231.
[12] Tanaka C, Kawai R, Rowland M. Dose-dependent pharmacokinetics of cyclosporin A in rats: events in tissues. Drug Metab Dispos 2000;28:582–589.
[13] Nestorov I. Whole body pharmacokinetic models. Clin Pharmacokinet 2003;42:883–908.
[14] Theil FP, Guentert TW, Haddad S, et al. Utility of physiologically based pharmacokinetic models to drug development and rational drug discovery candidate selection. Toxicol Lett 2003;138:29–49.
[15] Jones H, Parrott N, Jorga K, et al. A novel strategy for physiologically based predictions of human pharmacokinetics. Clin Pharmacokinet 2006;45:511–542.
[16] Dressman JB, Amidon GL, Reppas C, et al. Dissolution testing as a prognostic tool for oral drug absorption: immediate release dosage forms. Pharm Res 1998;15:11–22.
[17] Wu CN, Kou LF, Ma PQ, et al. Interspecies prediction of oral pharmacokinetics of different lacidipine formulations from dogs to human: physiologically based pharmacokinetic modelling combined with biorelevant dissolution. RSC Adv 2015;5:19844–19852.
[18] Hall ST, Harding SM, Evans GL, et al. Clinical pharmacology of lacidipine. J Cardiovasc Pharmacol 1991;17(Suppl. 4):S9–S13.
[19] Squassante L, Caveggion E, Braggio S, et al. A study of plasma disposition kinetics of lacidipine after single oral ascending doses. J Cardiovasc Pharmacol 1994;23:S94–S97.
[20] Lue BM, Nielsen FS, Magnussen T, et al. Using biorelevant dissolution to obtain IVIVC of solid dosage forms containing a poorly-soluble model compound. Eur J Pharm Biopharm 2008;69:648–657.
[21] Galia E, Nicolaides E, Hörter D, et al. Evaluation of various dissolution media for predicting in vivo performance of class I and II drugs. Pharm Res 1998;15:698–705.
[22] Yang B, Wu CN, Ji B, et al. The biorelevant concentration of Tween 80 solution is a simple alternative medium to the simulated fasted state intestinal fluid. RSC Adv 2015;doi:10.1039/c5ra17674c.
[23] Liu Y, Zhang MQ, Zhu JM, et al. Bioequivalence and pharmacokinetic evaluation of two formulations of glimepiride 2 mg: a single-dose, randomized-sequence, open-label, two-way crossover study in healthy Chinese male volunteers. Clin Ther 2010;32:986–995.
[24] Haidar SH, Makhlouf F, Schuirmann DJ, et al. Evaluation of a scaling approach for the bioequivalence of highly variable drugs. AAPS J 2008;10:450–454.
[25] Zhang X, Lionberger RA, Davit BM, et al. Utility of physiologically based absorption modeling in implementing quality by design in drug development. AAPS J 2011;13:59–71.