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In silico prediction of bioequivalence of Isosorbide Mononitrate tablets with different dissolution profiles using PBPK modeling and simulation

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ABSTRACT

Aim: The waiver of bioequivalence (BE) studies is well accepted for Biopharmaceutics Classification System (BCS) class I drugs in form of immediate-release solid oral products. This study aimed to assess whether the rapid dissolution profiles (≥85% in 30 min) was crucial to guarantee bioequivalence of isosorbide mononitrate (ISMN) and then established a clinically relevant dissolution specification (CRDS) for screening BE or non-BE batches.
Method: A physiologically based pharmacokinetic (PBPK) model was constructed by integrating clinical and non-clinical data by B^2O simulator. The model was verified by an actual clinical study (NMPA registration number: CTR20191360) with 28 healthy Chinese subjects. Then a virtual BE study was simulated to evaluate the bioequivalence of 7 virtual batches of ISMN tablets with different dissolution profiles, and the CRDS was established by integrating the results.
Result: The simulated PK behavior of ISMN was comparable to the observed. Even though the batches with slower dissolution were not equivalent to a rapid dissolution profile (≥85% in 30 min), it was demonstrated these batches would exhibit the similar in vivo performance. Meanwhile, the in vitro dissolution specification time point and the percentage of drug release (75% in 45 min) proved to have clinical relevance.
Conclusion: The virtual BE simulation by integrating in vitro dissolution profiles into the PBPK model provided a powerful tool for screening formulations, contributing to gaining time and reducing costs in BE evaluations.

1. Introduction

Early in 2000 the US Food and Drug Administration (FDA) released draft guideline for waiver of in vivo bioavailability and bioequivalence studies based on an Biopharmaceutics Classification System (BCS) (Kortejärvi et al., 2010), and this guideline was finalized in 2017 (Food and Drug Administration (FDA) 2017). Currently the biowaiver for immediate-release (IR) solid oral dosage forms containing BCS class I and III drug with high solubility, is well accepted by the National Medical Products Administration (NMPA), the European Medicines Agency (EMA), the Pharmaceuticals and Medical Devices Agency (PMDA) and other regulatory agencies (European Medicine Agency (EMA) 2010, World Health Organization (WHO) 2015, Brazilian Health Surveillance Agency (ANVISA) 2014). The BCS is a scientific framework for classifying drug substances based on their aqueous solubility and intestinal permeability. It allows for prediction of in vivo pharmacokinetics (PK) of IR solid oral products based on three major factors: dissolution, solubility, and intestinal permeability. For highly soluble and highly permeable drugs categorized as BCS class I, the rate and extent of absorption is unlikely dependent on drug dissolution and/or gastrointestinal transit time, theoretically the rate-limiting step is gastric emptying. For this purpose, in vitro dissolution data is required to demonstrate that the test and reference drug products are both rapidly dissolving (≥85% dissolved within 30 min) or very rapidly dissolving (≥85% dissolved within 15 min).

However, there are still some disputing questions for these drug

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products eligible for biowaiver: (1) Must the dissolution criterion be met to guarantee the bioequivalence for BCS class I drugs? It is known that dissolution criteria may vary from country to country, tighter requirement will be set somewhere. Taking BCS class I drug isosorbide mononitrate (ISMN) tablets as an example, the required dissolution rate is≥80% in 30 min, ≥80% in 15min and ≥85% in 15min according to the pharmacopoeia of China, America and Japan (Japanese Pharmacopoeia Commission 2016, Chinese Pharmacopoeia Commission 2015, U.S. Pharmacopoeia Commission 2017). Therefore, whether to take strictest dissolution rate to guarantee bioequivalence is a question. (2) Can in vitro dissolution and release behavior be considered as an indicator for predicting the pharmacokinetic (PK) behavior in human? In decades past, in vitro-in vivo correlation (IVIVC) has been employed as a surrogate for in vivo PK study and to support biowaiver, but the IVIVC rarely was established in consideration of drug disposition. Therefore, it is necessary to set up clinically relevant dissolution specification (CRDS) by linking in vitro dissolution data with in vivo PK behavior in humans. Recently several studies were performed to develop CRDS by in silico physiologically based pharmacokinetic (PBPK) modeling for orally administered drug products (Pepin et al., 2016, Loisios-Konstantinidis et al., 2020, Kato et al., 2020)).

PBPK approach is a key component of model-based drug development and is increasingly embraced by industry and regulatory authorities (Amitava, 2017, European Medicines Agency (EMA) 2018, Food and Drug Administration (FDA) 2018). The PBPK model considering ADME (absorption, distribution, metabolism, and excretion) behavior is desirable for predicting the human PK behavior accurately. Recently it has been recognized that PBPK model correlated with pharmaceutical tools in vitro such as dissolution can be applied to support virtual BE simulation (Pepin et al., 2016, Loisios-Konstantinidis et al., 2020, Babiskin and Zhang, 2015, Doki et al., 2017, Cristofoletti et al., 2017, Ibarra et al., 2018). In June 2020, FDA just released the information that virtual BE simulations were close to becoming standard (Food and Drug Administration 2020), accompanied with PBPK or big data–based method developments. Due to the accurate prediction for the PK behavior in vivo, in silico PBPK modeling can serve as a potential surrogate for clinical BE studies.

Isosorbide mononitrate (ISMN) is a long-acting anti angina drug by relaxing of vascular smooth muscle (Food and Drug Administration 2014). Compared with other nitric acid drugs, there is no first pass effect and it is well absorbed with a bioavailability of nearly 100% (Straehl and Galeazzi, 1985). This drug belongs to BCS class I drug with high solubility and high permeability (Li et al., 2011), however currently it is not listed as drug candidates for the waiver of in vivo bioequivalence studies by NMPA (National Medical Products Administration (NMPA) 2018, National Medical Products Administration (NMPA) 2018) . The objective of this article was to: (1) assess whether the rapid dissolution rate was essential to assure bioequivalence for BCS class I drug by taking ISMN as a case example; (2) establish a CRDS of ISMN tablets, using B^2O software, by in silico PBPK modeling, thereby providing a novel approach in BE evaluations.

2. Drugs and reagents

The ISMN reference tablets (20mg, lot 18229022A) were obtained from Lannett Co Inc (Pennsylvania, America), and the ISMN test tablets (20mg, lot V190401) were provided by Livzon Pharmaceutical Group Inc (Guangdong, China). Hydroxypropyl methylcellulose (HPMC), pH 1.2 hydrochloric acid (HCL) and pH 4 acetate buffer were commercially purchased from the Avantor Inc (Radnor, America).

Table 1
Input parameters used for the construction of PBPK model.

Parameter	Value	Source
Dosage form	Immediate Release Tablets	Reference (20)
Molecular weight (g/mol)	191.14	PubChem
log $P_{o:w}$	−0.15	Reference (28)
pKa	7.00	Reference (28)
Solubility (g/L) in water (25°C)	107.0	Reference (28)
Absorption		
$P_{eff,\ man}$ ($\times 10^{-4}$ cm/s)	6	Calculated using B^2O
Dissolution profile	Fig. 3	Experimental data
Distribution		
Distribution model	One compartment model	
Fraction unbound in plasma, fu	0.95	Reference (20)
Blood to Plasma ratio	0.825	From B^2O software
V_{ss} (L)	30	Calculated using B^2O
Elimination		
CL (L/h)	4	Calculated using B^2O

3. Method

3.1. Construction of in silico PBPK model

3.1.1. In vitro dissolution testing

The dissolution rate of ISMN reference and test tablets were determined in two dissolution media. 500 mL of HCL solution (pH 1.2) and well stirred pH 4 acetate buffer with 0.5% HPMC were used as the dissolution media to imitate the dissolution environment in fasting and fed conditions, respectively. The experiment was conducted at the temperature of 37±0.5°C and the speed of 50r/min according to the paddle method in Chinese Pharmacopoeia (2015 Edition) (Chinese Pharmacopoeia Commission 2015). The samples (10 mL) were withdrawn at 5, 10, 15, 30 and 45 min and an equivalent volume of fresh medium was added in time. The collected samples were filtrated and detected by a validated HPLC method. A Phenomenex Kinetex C18, 5μm, 4.6mm × 25cm analytical column was used; the mobile phase consisted of methanol-0.1% phosphoric acid solution (30:70% v/v); the column temperature was set at 40 °C and the flow rate at 0.8 mL/min. All experiments were repeated 12 times.

3.1.2. Construction and internal verification of in silico PBPK model

An in silico PBPK model was developed using B^2O simulator software (version 2.0, Hubei Yinghan Pharmaceutical Technology Co., Ltd, China) and the input parameters are summarized in Table 1. In B^2O

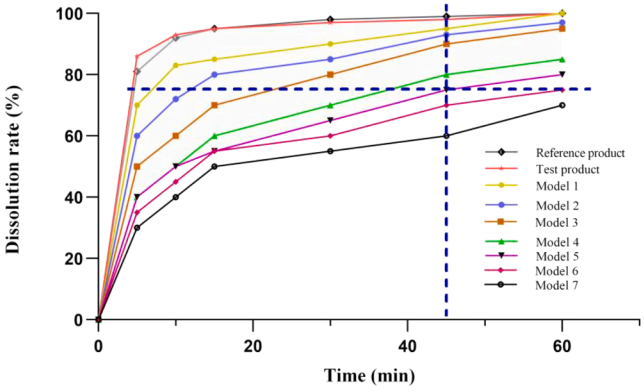

Fig. 1. Mean dissolution profiles in HCL (pH1.2) solution from the reference product, test product, and virtual batches (model 1-7).

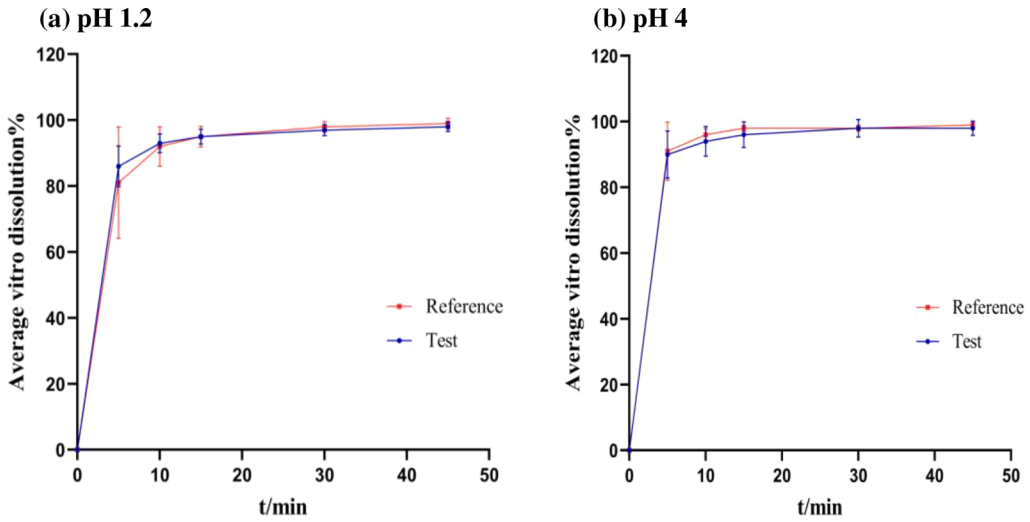

Fig. 2. The dissolution profiles (Mean ± Standard Deviation) of the reference and test products in two dissolution media (A: pH 1.2 HLC solution; B: well stirred pH 4 acetate buffer with 0.5% HPMC).

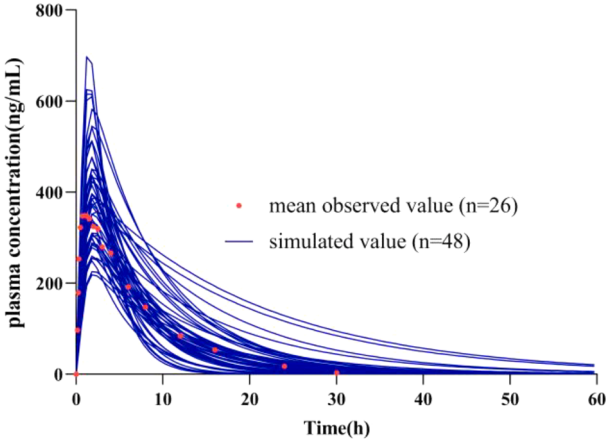

Fig. 3. The comparison between the simulated value (n=48) and observed value of reference product under fasting condition (Note: the observed data was obtained from literature and represented in the form of mean value).

simulator, the gastro-intestinal (GI) tract was divided into nine compartments anatomically, including one stomach, seven small intestinal compartments and one colon compartment, and provides physiologically based fluid flow rates, transit time and absorption rate for each compartment. The following assumptions were made: (1) the absorption in the stomach was insignificant compared with that in the small intestine. (2) the kinetics behavior including release, dissolution, permeability of ISMN drug through each segment of GI tract could be described by linear kinetics (Darwich et al., 2010). The absorption rate is mediated by the apparent permeability input and dissolution rate from solid to fluid, based on laboratorial data. The absorbed drug enters into different tissues in the body and connected in the circulating blood system. The dissolution profiles of ISMN reference and test tablets were obtained by in vitro dissolution tests. The effective permeability in humans ($P_{eff,man}$) and the post-absorptive parameters (i.e. volume of distribution at steady state (V_{ss}), and in vivo intravenous clearance (CL)) were estimated by simultaneously fitting to the observed data form literature (Teva. Isosorbide Mononitrate bioequivalence review 1997). Other input parameters, such as blood to plasma ratio (B:P ratio), molecular weight, $logP_{o:w}$, pKa, fraction unbound in plasma (fu) were acquired from the

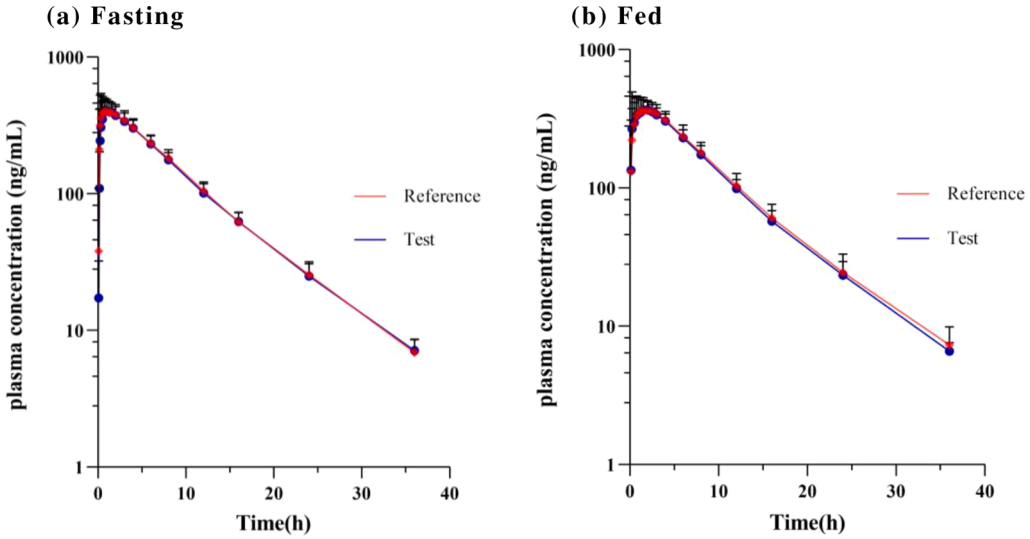

Fig. 4. The mean (±SD) plasma concentration-time profiles of the reference and test products under fasting and fed conditions ((a): the fasting condition; (b): the fed condition).

Table 2
Descriptive statistics of PK parameters of clinical study.

Parameter	Fasting (n=28)		Fed (n=28)	
	Test	Reference	Test	Reference
C_{max} (ng/mL)	454.27±105.07	486.58±136.38	450.23±133.98	417.21±83.88
AUC_{0-t} (h*ng/mL)	3556.82±491.02	3638.69±477.40	3449.39±548.59	3539.35±631.81
$AUC_{0-\infty}$ (h*ng/mL)	3682.08±470.10	3756.12±475.83	3554.27±531.11	3644.24±621.42
T_{max} (h)	1.00(0.25-2.00)	0.50(0.17-4.01)	1.25 (0.13-3.00)	1.25(0.13-4.00)
$t_{1/2}$ (h)	5.66±0.46	5.66±0.50	5.49±0.60	5.55±0.61

Note: T_{max} is represented by median (minimum, maximum), and other parameters are represented by arithmetic Mean (±standard deviation, SD).

Table 3
The result of BE analysis of clinical study.

Parameter	Fasting (n=28)		Fed (n=28)	
	Geomean Ratio (T/R)	90% confidence interval (%)	Geomean Ratio (T/R)	90% confidence interval (%)
C_{max} (ng/mL)	94.82	88.18- 101.97	107.44	98.60- 117.08
AUC_{0-t} (h*ng/mL)	98.58	97.03- 100.15	99.00	96.75- 101.30
$AUC_{0-\infty}$ (h*ng/mL)	98.92	97.52- 100.34	98.94	96.61- 101.34

Note: T represented the test product and R represented the reference product.

Table 4
Descriptive statistics of the PK parameters under the fasting and fed conditions after oral ISMN administration in virtual Chinese subjects.

Sample size	Parameter	Fasting		Fed	
		Test	Reference	Test	Reference
n=12	T_{max}(h)	1.86 (1.78,2.18)	1.87 (1.78,2.20)	2.00 (1.72,2.48)	2.00 (1.70,2.46)
	C_{max}(ng/ml)	385.85	384.16	389.96	388.65
	AUC_{0-t}(h*ng/ml)	3731.53	3709.44	3153.57	3142.55
n=24	T_{max}(h)	1.85 (1.34,2.30)	1.87 (1.36,2.32)	2.06 (1.70,2.40)	2.05 (1.70,2.38)
	C_{max}(ng/ml)	435.30	438.68	418.47	413.18
	AUC_{0-t} (h*ng/ml)	3720.11	3751.20	3632.41	3593.46
n=48	T_{max}(h)	1.79 (1.24,2.28)	1.79 (1.22,2.64)	2.04 (1.18,2.50)	2.06 (1.20,2.48)
	C_{max}(ng/ml)	513.61	513.94	402.69	402.39
	AUC_{0-t} (h*ng/ml)	3803.02	3807.85	3471.36	3449.39

Note: Tmax is represented by median (minimum, maximum), and other parameters are represented by arithmetic Mean.

literature and public databases (Li et al., 2018, Wang et al., 2019).

Demographic variables such as sex, age and weight are treated as covariates of clearance and volume. The inter-subject variability is introduced within the above parameters to generate virtual human population and PK profiles. Moreover, a B^2O Bootstrap method is applied to the PK parameters (AUC and C_{max}) to further introduce the intra-subject variability. Therefore, it is possible to separate the attribution from inter and intra variability, which could be advantageous to visualize highly variable compounds. In this article, default value (30%) in B^2O simulator was implemented for inter-subject variability. In order to make an internal verification for the PBPK model, a virtual BE study using Chinese population (n=48) under the fasting condition was simulated, and intra-subject coefficient of variation (CV%) was set as 10%. The PK profiles of reference product were compared with the observed data from literature (Teva. Isosorbide Mononitrate bioequivalence review 1997).

3.2. External verification of the PBPK model

3.2.1. Clinical study

A single-dose, two-period, two-sequence, cross-over clinical BE study under fasting and fed conditions was conducted (NMPA registration number: CTR20191360). The study was approved by the Ethical Committee of Wuxi People's hospital (Jiangsu, China). The study was conducted in accordance with Good Clinical Practice regulations and the ethical principles stated in the Declaration of Helsinki. All the subjects signed the informed consent in person.

Fifty-six healthy Chinese subjects with age of 19-44 years, body weight of 46.0-78.8 kg, and average body mass index of 19.4-26.0 kg/m^2 were enrolled, twenty-eight (20 male and 8 female subjects) of them participated in fasting BE study, and the others (20 male and 8 female subjects) participated in fed BE study. All the subjects were in good health without significant diseases according to medical history and physical examinations. Under both fasting and fed conditions, subjects were randomized into two groups and each group were orally administrated two drug treatments at two different periods with a 7-day washout time.

Serial blood samples under fasting condition (4mL at each time point) were collected as following: 0 (pre-dose), 5, 10, 15, 20, 30, 45min, 1, 1.25, 1.5, 2, 3, 4, 6, 8, 12, 16, 24, and 36h post-dose. The blood sampling points under fed condition were taken as following: 0 (pre-dose) 8, 15, 30, 45min, 1, 1.25, 1.5, 2, 2.5, 3, 4, 6, 8, 12, 16, 24, and 36h post-dose. A validated HPLC-MS/MS method was utilized to determine the plasma concentrations (Zhou et al., 2020). PK parameters were calculated using non-compartmental analysis (NCA) method in WinNonLin (Version 8.1, Pharsight, NC, USA). C_{max} was the maximum plasma concentration in a profile and T_{max} was the time to C_{max}. The elimination half-life ($t_{1/2}$) was calculated using the formula ($t1/2=Ln(2)/\lambda_z$). λ_z was the first-order rate constant of terminal elimination phase and calculated by linear regression of log-transformed concentration-time data with at least three concentration points included. The area under the curve from time zero to time t (AUC_{0-t}) was calculated based on the Linear Up Log Down method. The AUC from time zero to infinity ($AUC_{0-\infty}$) was estimated as $AUC_{0-t}+C_t/\lambda_z$, where C_t was the plasma concentration of the last measurable sample. If the 90% confidence intervals (CI) of C_{max}, AUC_{0-t}, $AUC_{0-\infty}$ geomean ratio (GMR) fell within the bioequivalence criteria (80.00%-125.00%), the test product could be considered as bioequivalent to reference product.

3.2.2. In silico simulation

In silico simulations with CV=10% and different sample sizes (n=12,24,48) were performed to predict the bioequivalent risk of ISMN test tablets under fasting and fed conditions. Based on B^2O Chinese population database, the virtual healthy Chinese subjects were generated with age of 20-60 years, body weight of 50-100 kg, and a proportion of females of 0.5. The trial design was identical to the clinical BE

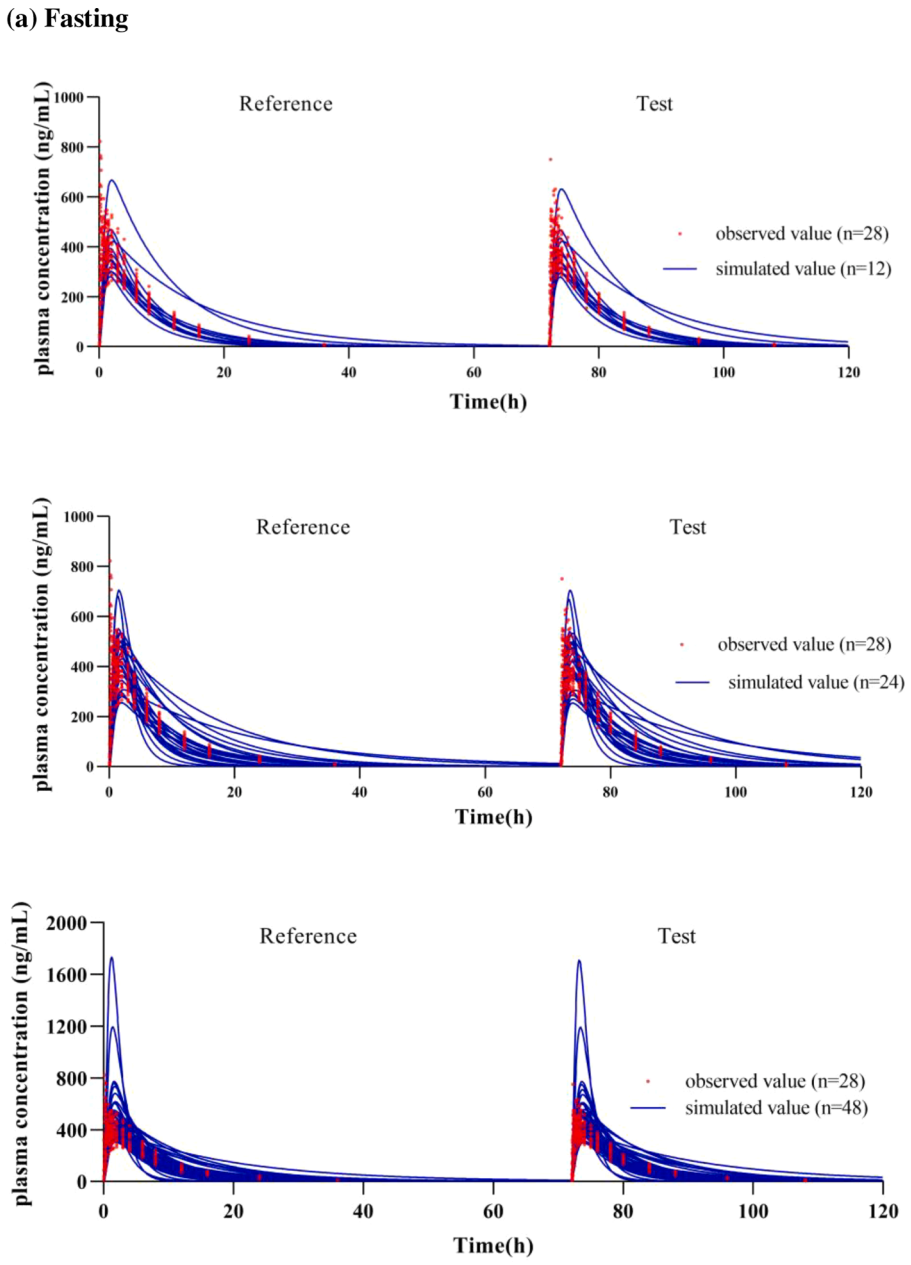

Fig. 5. The comparison between simulated PK profiles (n=12, 24, 48) and observed PK profiles (n=28) ((a): the fasting condition; (b): the fed condition).

trial, a single dose study of random, two periods, two sequence, cross-over controlled. The simulated plasma concentration-time profiles and pharmacokinetic (PK) parameters were compared with observed value of clinical study to verify the PBPK model.

3.3. In silico prediction of bioequivalence of ISMN tablets with different dissolution profiles

After the construction and verification of the PBPK model, a virtual BE study under fasting condition was simulated to explore whether the rapid dissolution rate (≥85% in 30 min) was essential to assure the bioequivalence of ISMN tablets. Besides the reference and test (Section 2), additional 7 virtual tablet batches (model 1-7) with gradually decreasing dissolution rate at HCL solution (pH 1.2) were generated through B^2O simulator. The reference, test, model 1, model 2, model 3, model 4, model 5, model 6 and model 7 products were dissolved 98%, 97%, 90%, 85%, 80%, 70%, 65%, 60% and 55% within 30 min, respectively (Fig. 1). The virtual BE study with a sample size of 24 and CV% value of 10% was simulated by integrating these dissolution profiles into PBPK model. If simulated PK parameters met the bioequivalence criteria (see Section 3.2.1), the virtual batch could be considered as bioequivalent.

3.4. Establishment of clinically relevant specification

The dissolution specification limit is defined by a Q value (i.e. the percentage of drug release) at a given time point, which allows discrimination between acceptable and non-acceptable batches. The virtual BE result of above 7 virtual batches was used to find the Q value and time point by comparing with reference product.

However, virtual BE simulations in Section 3.3 was only performed with a sample size of 24 and CV% value of 10%. According to the clinical trials after orally administrated 20mg ISMN tablets published in PMDA and FDA (Teva. Isosorbide Mononitrate bioequivalence review 1997,

(b) Fed

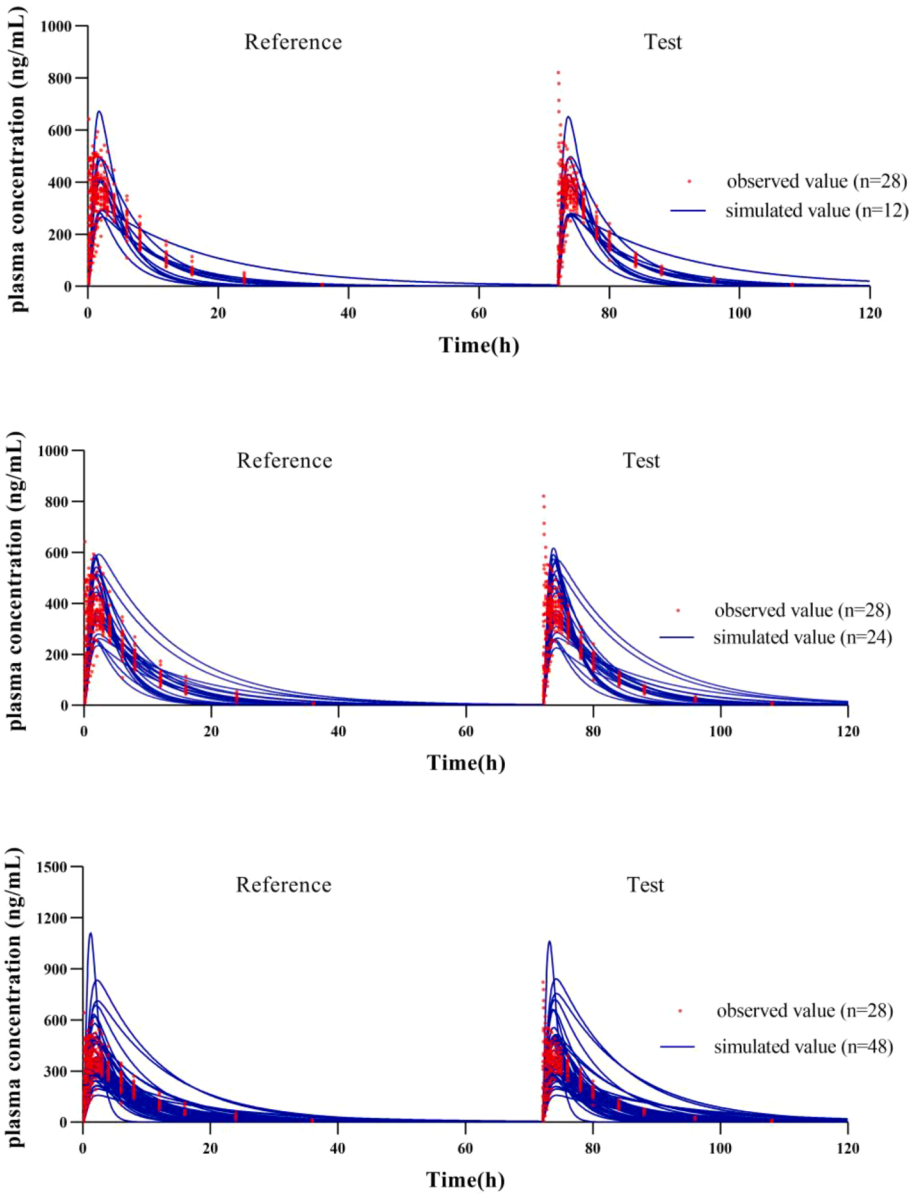

Fig. 5. (*continued*).

Table 5
The absolute predictive error (%PE) values between observed and simulated PK parameters under the fasting and fed conditions.

No.		Fasting			Fed		
		C_{max}	AUC_{0-t}	T_{max}	C_{max}	AUC_{0-t}	T_{max}
1	Test	15.06	-4.91	-86.00	13.39	8.58	-60.00
	Reference	21.05	-1.94	-274.00	6.85	11.21	-60.00
2	Test	4.18	-4.59	-85.00	7.05	-5.31	-64.80
	Reference	9.84	-3.09	-274.00	0.97	-1.53	-64.00
3	Test	-13.06	-6.92	-79.00	10.56	-0.64	-63.20
	Reference	-5.62	-4.65	-258.00	3.55	2.54	-64.80

Note: (1) The predicted value with sample size of 12,24,48 was compared with observed of clinical trial (n=28) respectively and named No.1, No.2 and No.3; (2) %PE = [(Observed value – Simulated value) / Observed value] × 100%. C_{max} and AUC_{0-t} were compared in the form of arithmetic Mean; T_{max} was compared in the form of median.

Pharmaceuticals and Medical Devices Agency (PMDA) 2014), the CV% value of C_{max} under fasting condition was calculated to between 9.8%-18.1% and that of AUC_{0-t} was between 8.17%-18.4%. These data suggested that ISMN was not a highly variable drug with a maximum CV % value of 18.4% (Center for Drug Evaluation 2018). In order to further establish the CRDS, a higher CV% value of 20% was taken into consideration, which could cover the whole intra-subject variability range of ISMN tablets. Therefore, bootstrap analysis method was applied to adjust the CV% values (CV%=10%, 20%) under different sample sizes (n=12, 24, 48) and virtual BE simulations under fasting condition were performed. Then a safe dissolution space where the dissolution profiles were anticipated bioequivalence could be discovered based on the virtual BE result and the borders corresponding to the safe space could be used to establish the CRDS.

Table 6
The simulation result of virtual BE study of ISMN tablets with different dissolution profiles.

	Reference product			Test product and virtual batches			GMR (%) (Test/Reference)		90% CI (%)	
	AUC_{0-t} (h*ng/ml)	C_{max} (ng/ml)	T_{max} (h)	AUC_{0-t} (h*ng/ml)	C_{max} (ng/ml)	T_{max} (h)	$RAUC_{0-t}$	RC_{max}	$RAUC_{0-t}$	RC_{max}
Reference *vs* test	3553.09	416.27	1.83 (1.35-2.31)	3570.09	417.99	1.83 (1.44-2.40)	100.48	100.41	98.97-102.01	98.93-101.92
Reference *vs* model 1	3356.59	419.58	1.83 (1.20-2.55)	3311.66	414.65	1.83 (1.35-2.65)	98.66	98.83	96.42-100.96	96.58-101.13
Reference vs model 2	3357.36	419.87	1.92 (1.44-2.31)	3351.53	419.98	1.83 (1.35-2.16)	99.83	100.03	97.03-102.71	97.23-102.90
Reference vs model 3	3046.89	401.99	1.83 (1.44-2.16)	2.97461	393.73	1.83 (1.59-2.07)	97.63	97.95	95.37-99.94	95.67-100.27
Reference vs model 4	3359.29	360.07	1.92 (1.35-2.40)	3.06366	329.47	1.92 (1.44-2.40)	91.2	91.5	89.13-93.31	89.43-93.63
Reference vs model 5	2638.91	349.22	1.83 (1.44,2.31)	2308.09	306.72	1.83 (1.58,2.16)	87.46	87.83	83.92-91.16	84.27-91.54
Reference vs model 6	3079.63	399.1	1.83 (1.44,2.40)	2602.05	338.62	1.83 (1.35,2.31)	84.49	84.85	82.43-86.61	82.77-86.98
Reference vs model 7	3575.75	383.82	1.92 (1.44-3.75)	2918.93	314.7	1.92 (1.59-3.85)	81.63	81.99	77.91-85.53	78.27-85.88

Note: (1) The test product (Livzon Pharmaceutical Group Inc, lot V190401) and 7 virtual tablet batches (model 1-7) with different dissolution profiles were compared with the reference product respectively.
(2) T_{max} was represented in the form of median (minimum, maximum) and other parameters were represented in the form of geometric mean.

4. Result

4.1. Construction and internal verification of in silico PBPK model

The dissolution profiles of reference and test products were used as input parameters (Fig. 2). Since the observed data of reference product from literature was used to construct the PBPK model, a virtual BE study (n=48) under the fasting condition was conducted and compared with the observed from literature to make the internal verification of PBPK model (Teva. Isosorbide Mononitrate bioequivalence review 1997). As shown in Fig. 3, the observed mean PK profiles of reference product from literature were in good agreement with the simulated PK profiles, which preliminarily suggested the PBPK model have an accurate predictive ability of in vivo performance of ISMN tablets.

4.2. External verification of in silico PBPK model

4.2.1. BE evaluation of clinical study

The mean plasma concentration-time profiles and PK parameters of clinical study are shown in Fig. 4 and Table 2. The result of BE analysis is displayed in Table 3, indicating that the 90% CIs of PK parameters (C_{max}, AUC_{0-t}, $AUC_{0-\infty}$) under fasting condition were within 80.00%-125.00% and the same was true of the fed BE study. Therefore, it was concluded that the test product was bioequivalent to the reference product.

4.2.2. External verification of in silico PBPK model

The virtual BE study in healthy Chinese subjects after oral administration of ISMN tablets was performed. The simulated PK parameters (T_{max}, C_{max}, AUC_{0-t}) is summarized in Table 4. The in silico PBPK model was externally verified by comparison with clinical study. The simulated PK profiles (n=12, 24, 48) were well consistent with observed values after orally administrated ISMN tablets in the fasting and fed conditions (Fig. 5). As shown in Table 5, the prediction error (%PE) values between simulated and observed data of C_{max} and AUC_{0-t} were within 21.05%. The %PEs for median T_{max} were within 274%. However, under the fasting condition, all simulated T_{max} values (1.22 - 2.64h) were within the range of 0.17-4.01h of the observed. With regard to the data under fed condition, all simulated T_{max} values (1.18-2.50 h) were within the range of 0.13-4.00h of the observed value (Table 2 and 4). These analysis demonstrated that the simulated PK parameters were in good agreement with observed values (Food and Drug Administration 1997). The PBPK model could recover the PK profiles well in healthy Chinese subjects after orally administrated ISMN tablets.

4.3. In silico prediction of bioequivalence of ISMN tablets with different dissolution profiles

A total 7 virtual ISMN tablet batches with different dissolution profiles were generated to conduct virtual BE study under the fasting condition. As shown in Table 6, there was a gradual downward trend in the geometric mean of AUC_{0-t} and C_{max} with the decrease of dissolution rate, the same with the trend of 90% CI. The 90% CIs of AUC_{0-t} and C_{max} ratio of model 7 were 77.91%-85.53% and 78.27%-85.88% respectively, indicating that model 7 was not bioequivalent to the reference product. However, the 90% CI values of model 1-6 were all within the accepted bioequivalence criterion, even if model 2-6 failed to meet the rapid dissolution criterion. Therefore, there were enough confidence in the hypothesis that the rapid dissolution rate (≥85% dissolved within 30 min) wasn't essential to assure bioequivalence for ISMN tablets.

4.4. Establishment of clinically relevant specification

According to the result of Section 4.3, model 6 was predicted to be bioequivalent in virtual BE study with CV% value of 10% and sample size of 24, but still had the risk to exceed the bioequivalent interval. In order to further find the most appropriate dissolution profile used for the border set of dissolution safe space, the virtual BE study with different CV% values (CV%=10%, 20%) and different sample sizes (n=12, 24, 48) was performed.

When horizontally comparing (a)-(h) in Fig. 6, it was found that with the decrease of dissolution rate, the 90%CI (i.e. the shadow area) moved downward and gradually approached the lower bioequivalent limit until the model 6 (CV%=10%, n=12) and model 7 exceeded the bioequivalent interval. In the strict sense, though model 5 sat comfortably within a region of dissolution performance where bioequivalence is anticipated, the lower limit of 90% CI was near an edge of bioequivalence interval, indicating that tablet batches dissolved slower than model 5 had risk of exceeding bioequivalence interval. In other words, the dissolution standard of model 5 (dissolved 75% in 45 min) had the potential to detect and reject non-BE batches. Therefore, the dissolution profile of model 5 could be set as the border of dissolution safe space (i.e. the gray shaded area in Fig. 1) and used to set CRDS to minimize the risk of bioequivalence failure. In addition, for the establishment of CRDS, the recent paper published by Committee for Medicinal Products for Human

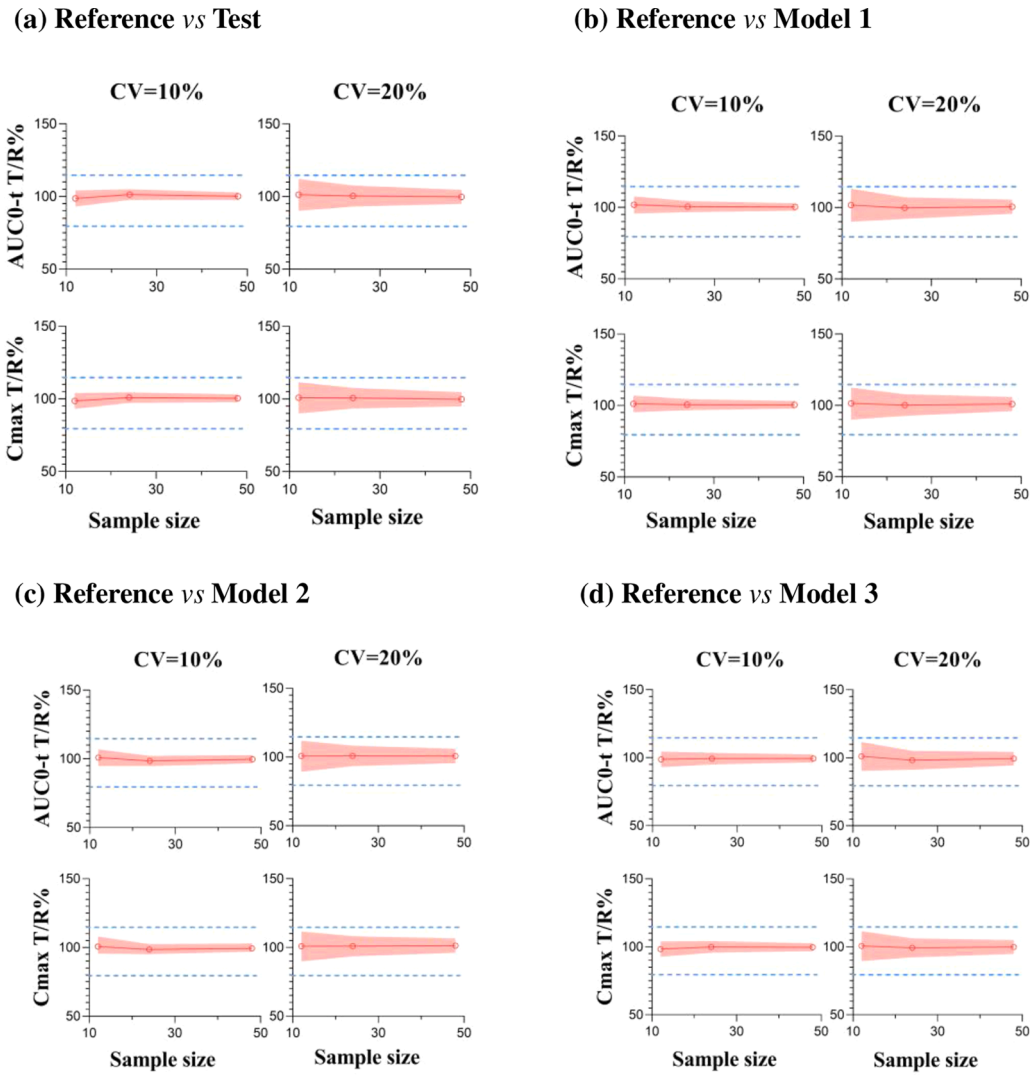

Fig. 6. The BE result of virtual tablet batches (model 1-7) and test (T) product compared with reference (R) product with different sample sizes and intra-subject coefficients of variation (CV%) under fasting condition (Note: the dotted line: the bioequivalence interval of 80.00%-125.00%; the red line: the geometric mean value of C_{max} T/R% or AUC_{0-t} T/R%; the shadow: 90% CI).

Use (CHMP) recommended that Q value was usually set as Q = 75%, 80%, or 85% in 15/30/45 min (European Medicines Agency (EMA) 2017). Accordingly, a CDRS with Q value of 75% and time point of 45 min in HCL (pH 1.2) medium was established based on model 5 (Fig. 1).

5. Discussion

This article explored whether the rapid dissolution rate was necessary to guarantee bioequivalence for BCS class I drug and further established a clinically relevant dissolution specification for screening the BE and non-BE batches.

For this purpose, an in silico PBPK model integrated with in vitro dissolution test was constructed. The establishment of in vitro-in vivo correlation (IVIVC) was critical to realize the prediction of in vivo performance by in vitro pharmaceutical tools (Heimbach et al., 2019, Suarez-Sharp et al., 2018). For ISMN compound classified as BCS I drug, dissolution and permeation processes were not rate-limiting steps. It is noted that dissolution test and permeation test are two main pharmaceutical tools in vitro, but the former are usually applied to develop the IVIVC (Heimbach et al., 2019). Moreover, the permeation parameter (i.e. $P_{eff,man}$) could be estimated by simultaneously fitting to the observed data from literature in B^2O software [27]. Thus in vitro dissolution test was conducted to establish the IVIVR and the $P_{eff,man}$ parameter was estimated by B^2O simulator. As for the selection of dissolution media, since T_{max} values of the reference product were 0.8 ± 0.6 h and 1.85 ± 0.93 h under the fasting/fed conditions according to literature record, respectively (Teva. Isosorbide Mononitrate bioequivalence review 1997), it could be speculated that most of the absorption could be completed within the time range of gastric emptying and ISMN tablets mainly dissolved in the stomach. In this regard, HCL (pH 1.2) solution and pH 4 acetate buffer were taken as the dissolution media to imitate the dissolution environment of ISMN tablets in fasting and fed conditions, respectively (González-García et al., 2015). The HPMC (a viscosity-enhancer) was added into the pH 4 acetate buffer to simulate the effect of food on dissolution rate (Siow et al., 2019).

In B^2O simulator, the absorption of ISMN compound is a multi-compartment GI transit model integrated with human population parameters based on Monte Carol simulation. As for the in vivo disposition process of ISMN, since BE study focus on the release of a drug substance from a drug product and subsequent absorption into systemic circulation, the active ingredients form test and reference products are considered to be absent of difference in disposition process. Accordingly,

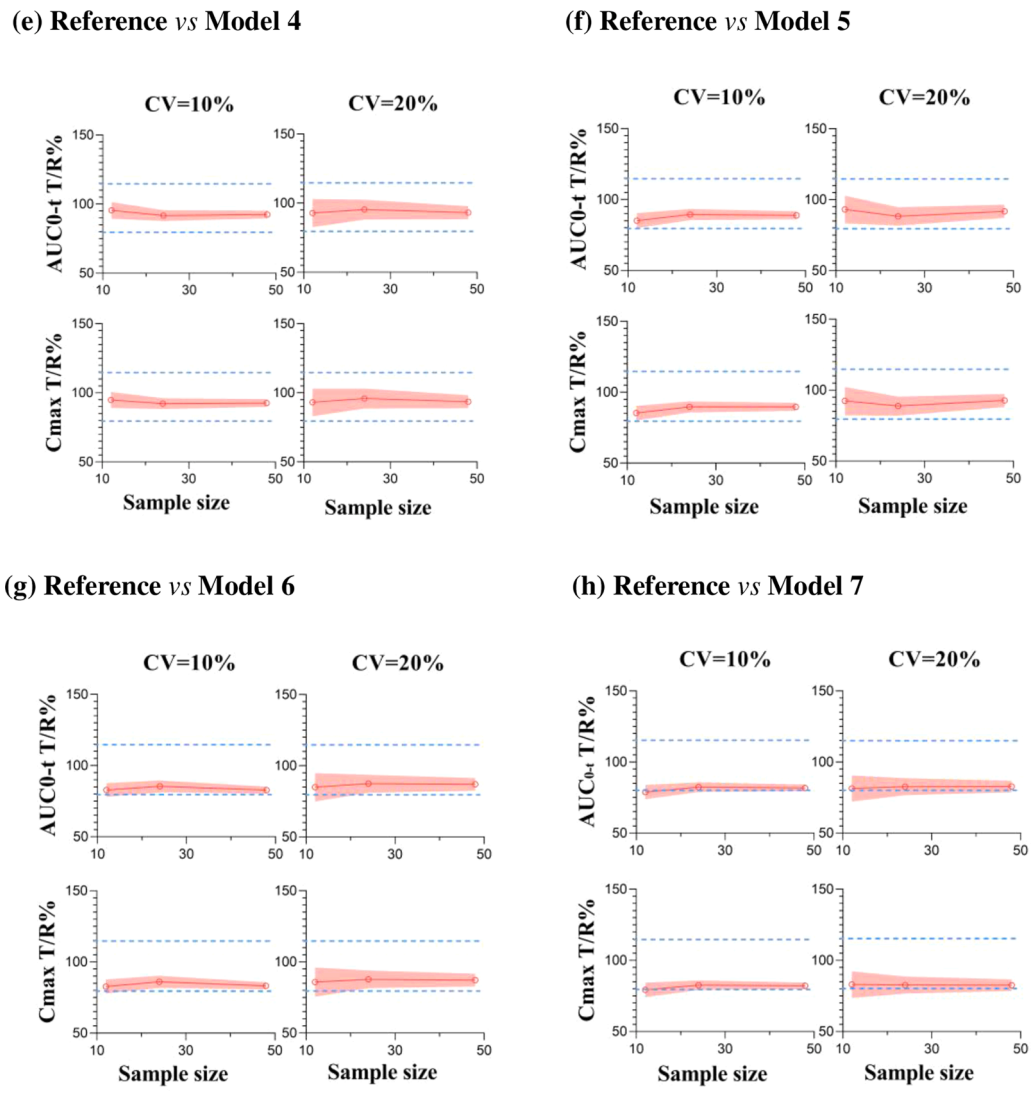

Fig. 6. (*continued*).

the disposition part of PBPK model was constructed using V and CL parameters estimated by simultaneously fitting to the observed data form literature (Teva. Isosorbide Mononitrate bioequivalence review 1997).

The prediction ability of PBPK model was successfully verified by comparison with the observed data from literature and clinical study. Once enough confidence in the predictive ability of PBPK model, virtual BE simulation was performed to predict the bioequivalence of ISMN tablets with different dissolution profiles. FDA recommendations for the biowaiver of BCS class I drug state that the IR drug product is considered rapidly dissolving when ≥85% of the labeled amount dissolves within 30 minutes in 0.1 M HCL, pH 4.5 and pH 6.8 buffers. Though HCL (pH1.2) medium was only taken in this article, the virtual tablet batches with slower dissolution rate were demonstrated to be bioequivalent to reference product, suggesting that rapid dissolution rate was necessary to guarantee bioequivalence for BCS class I drug. Furthermore, virtual BE simulations under different CV% values and sample sizes were performed to search the borders of dissolution safe space where bioequivalence is anticipated, and establish a clinically relevant dissolution specification for screening BE and non-BE batches. Under this approach, the border of dissolution safe space was set strictly based on model 5. An in vitro dissolution specification (Q value) of "Q=75% in 45 min" in HCL (pH 1.2) medium was finally established for ISMN tablets, which demonstrated that a wider dissolution range could meet bioequivalence criterion.

Currently, the biowaiver based on BCS classification is only realized in a part of BCS I and III drugs with restrict requirements. In contrast, there are no restriction of BCS classification on the construction of PBPK model, which could also be used for BCS II and IV drugs. Moreover, compared with conventional IVIVC method, PBPK model offers enhanced mechanistic insight into the ADME behavior (especially the absorption process) and the correlation between in vitro and in vivo performance (Kato et al., 2020). Given the precise prediction of PK behavior, virtual BE simulation based on PBPK model provide another selection for bioequivalence evaluation. CRDS could also be established using PBPK model, to support a wider dissolution acceptance criterion for commercial drug batches (Food and Drug Administration 2018).

In this article, ISMN (BCS class I drug) was taken as the case example. Future work could investigate the bioequivalence risk and clinically relevant specification of BCS II - IV drugs based on virtual BE simulation. In this way, the model informed drug development and regulatory flexibility will be greatly improved.

6. Conclusion

An in silico PBPK model was successfully developed and verified by literature and clinical study. Through virtual BE simulation, virtual ISMN tablet batches that failed to meet the rapid dissolution criterion

were demonstrated to be bioequivalent. A clinically relevant dissolution specification to detect and reject non-BE batches was further established. Overall, the virtual BE study based on in silico PBPK modeling suggests a promising and powerful tool to predict anticipated clinical outcomes and explore the clinically relevant dissolution specification of drug product.

Author contributions

Fan Zhang and Ranran Jia performed the model simulation; Yingping Zhou, Ni Wu and Aijing Liu performed the bioassay; Bo Liu, Xiang Ye and Zhou Zhou provided assistance in PBPK modeling; Haitang Hu and Zhihui Han provided support of in vitro dissolution data and drug information; Qing He and Ying Ding completed the BE clinical study; Hongyun Wang designed and directed the whole project.

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