



Virtual bioequivalence for achlorhydric subjects: The use of PBPK modelling to assess the formulation-dependent effect of achlorhydria



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ABSTRACT

Majority of bioequivalence studies are conducted in healthy volunteers. It has been argued that bioequivalence may not necessarily hold true in relevant patient populations due to a variety of reasons which affect one formulation more than the other for instance in achlorhydric patients where elevated gastric pH may lead to differential effects on formulations which are pH-sensitive with respect to release or dissolution. We therefore examined achlorhydria-related disparity in bioequivalence of levothyroxine and nifedipine formulations using virtual bioequivalence within a physiologically-based pharmacokinetic (PBPK) modelling framework. The *in vitro* dissolution profiles at neutral pH were incorporated into PBPK models to mimic the achlorhydria with *in vitro-in vivo* relationship established using bio-relevant pH media. The PBPK models successfully reproduced the outcome of the bioequivalence studies in healthy volunteers under the normal conditions as well as under proton pump inhibitor-induced achlorhydria. The geometric mean test/reference ratios for C_{max} and AUC between levothyroxine tablet and capsule in patients receiving proton pump inhibitor were 1.21 (90%CI, 1.13–1.29) and 1.09 (90%CI, 1.02–1.17), respectively. Extension of the virtual bioequivalence study to Japanese elderly, who show high incidence of achlorhydria, indicated bio-inequivalence which C_{max} and AUC ratios between nifedipine control-released reference and test formulations were 3.08 (90%CI, 2.81–3.38) and 1.57 (90%CI, 1.43–1.74), respectively. Virtual bioequivalence studies through the PBPK models can highlight the need for conduct of specific studies in elderly Japanese populations where there are discrepancies in pH-sensitivity of dissolution between the test and reference formulations.

1. Introduction

A generic pharmaceutical product is marketed if it is therapeutically equivalent to the corresponding reference product containing the same active pharmaceutical ingredient (Davitt et al., 2013). Therapeutic equivalence is assumed if the concentration-time profiles are similar. The same criteria is applied during the development of any proprietary drug product when for variety of reasons the formulation is changed between early phase clinical studies and later studies prior to getting the drug into market. The pharmaceutical companies must demonstrate that the rate and extent of absorption from the new formulation is not significantly different from that of the reference formulation. Clinical studies to establish bioequivalence between two formulations are generally conducted in young healthy volunteers. The debate over the conduct of bioequivalence studies in patients as opposed to healthy volunteers is not a new one (Klintmalm, 2011; Morihara et al., 2001). However, the possibility to conduct “virtual bioequivalence” using *in*

silico modelling of the target population is a new concept materialized with the advent of mechanistic models of oral drug absorption which combines *in vitro* information with the physiologically-based pharmacokinetic (PBPK) models to postulate *in vivo* consequences of any differences between formulations not only in healthy volunteers but in variety of other populations who are not typically assessed as part of the bioequivalence studies (Cristofolletti et al., 2017).

In the current study we have used achlorhydria as an example of attributes for gastro-intestinal tract that might have different incidence in the target population compared to the healthy volunteer populations which may cause disparities in the conclusions drawn regarding the bioequivalence in the two populations. Achlorhydria is defined as a state of the absence of hydrochloric acid in gastric juices. The prevalence of achlorhydria increases with age, and > 70% of Japanese elderly develop gastric hypoacidity (Morihara et al., 2001). Elevated gastric pH in achlorhydric elderly may affect bioequivalence between drug formulations where pH-sensitivity for *in vitro* dissolution differs.

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Administration of proton pump inhibitors induces gastric hypoacidity and also has the possibility to produce unacceptable results of bioequivalence between such drug formulations (Seng et al., 2015).

Several generic formulations of drugs such as levothyroxine and nifedipine show different pH-sensitivity of *in vitro* dissolution to the corresponding reference formulation (Garbacz et al., 2009; Pabla et al., 2009; Schug et al., 2002a, 2002b; Wonnemann et al., 2008). Levothyroxine, L-form of thyroid hormone thyroxine used for the treatment of hypothyroidism, is orally administered as tablets or a soft gelatin capsule where pH-sensitivity for *in vitro* dissolution differs between commercial products. Levothyroxine dissolves slowly from tablet at mild acidic and neutral pH compared to strong acidity, whereas soft gelatin capsule containing levothyroxine dissolved in glycerin shows a consistent dissolution profile without pH-dependency (Pabla et al., 2009). Once-daily tablet formulations of nifedipine, a calcium channel blocker used for the treatment of hypertension and angina, are marketed as control-released (CR) formulations where the release system differs such as oral osmotic push-pull system (OROS) and hydrophilic matrix tablets (Garbacz et al., 2009). An OROS tablet of nifedipine CR provides pH-independent dissolution profiles, while the corresponding hydrophilic matrix tablet has obvious pH-dependency of *in vitro* dissolution (Garbacz et al., 2009). Different pH-sensitivity of *in vitro* dissolution between these formulations raises issues concerning the possibility that bioequivalence cannot be necessarily assumed the same in healthy volunteer and achlorhydric patients population. The aim of study is to examine achlorhydria-related disparity in bioequivalence of levothyroxine and nifedipine CR formulations using virtual bioequivalence within PBPK modelling framework including *in vitro*–*in vivo* correlation (IVIVC) modelling.

2. Materials and methods

A workflow for virtual bioequivalence studies using the PBPK

models that were applied to postulate *in vivo* consequences of any differences between formulations is outlined in Fig. 1.

2.1. Formulations

The levothyroxine sodium reference tablet and test capsule formulations used for virtual bioequivalence studies were Synthroid® (Abbott Laboratories, IL, USA) and Tirosint® (IBSA Institut Biochimique SA, Switzerland), respectively. The nifedipine CR reference and test formulations used for virtual bioequivalence studies were Adalat® OROS (Bayer AG, Germany) and Nifedipine Coral® (So.Se.PHARM S.r.l., Italy), respectively. *In vitro* dissolution profiles of the reference and test formulations for levothyroxine and nifedipine were obtained from the literature (Garbacz et al., 2009; Pabla et al., 2009). The dissolutions for levothyroxine formulations were carried out in dissolution medias containing 0.05% sodium lauryl sulfate, which were 0.1 N hydrochloric acid representing pH 1.2 and 0.05 M ammonium acetate buffers by adjusting the pH with acetic acid or ammonium hydroxide to 5.0, 6.0, or 7.0 (Pabla et al., 2009). The dissolutions for nifedipine CR formulations were carried out in USP simulated gastric fluid without enzymes pH 1.2 with 1% SDS, USP acetate buffer pH 4.5 with 1% SDS, and USP buffer solution for nifedipine extend release tablets pH 6.8 (Garbacz et al., 2009). These dissolution profiles were digitized using GetData Graph Digitizer version 2.26 (Fig. 2). Plasma levothyroxine concentration profiles with or without intravenous administration of esomeprazole and plasma nifedipine concentration profiles under fasted and fed state were also obtained from the literature and digitized (Colucci et al., 2011; Schug et al., 2002b; Seng et al., 2015).

2.2. PBPK model development

PBPK modelling and simulation was employed using the Simcyp® Simulator (V16.1; Certara, Sheffield, UK). PBPK model was developed

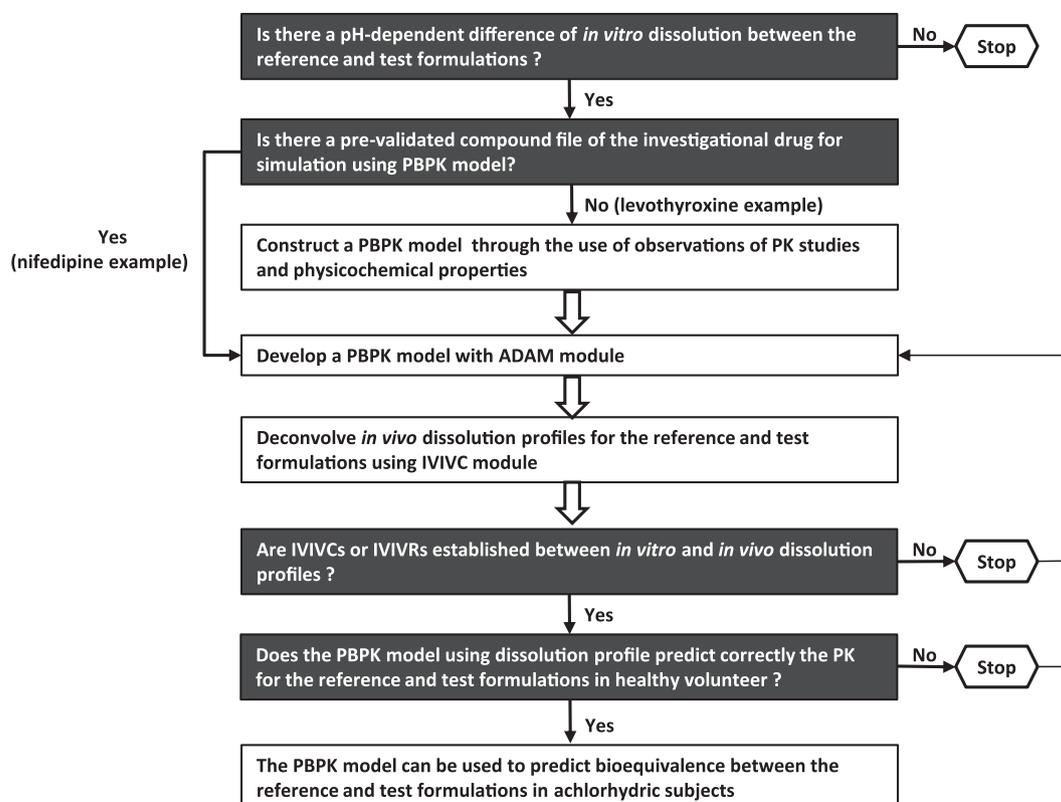


Fig. 1. Applied workflow for virtual bioequivalence studies using the physiologically-based pharmacokinetic (PBPK) models to postulate *in vivo* consequences of any differences between formulations. PK, pharmacokinetic; ADAM, Advanced Dissolution, Absorption and Metabolism; IVIVC, *in vitro*–*in vivo* correlation; IVIVR, *in vitro*–*in vivo* relationship.

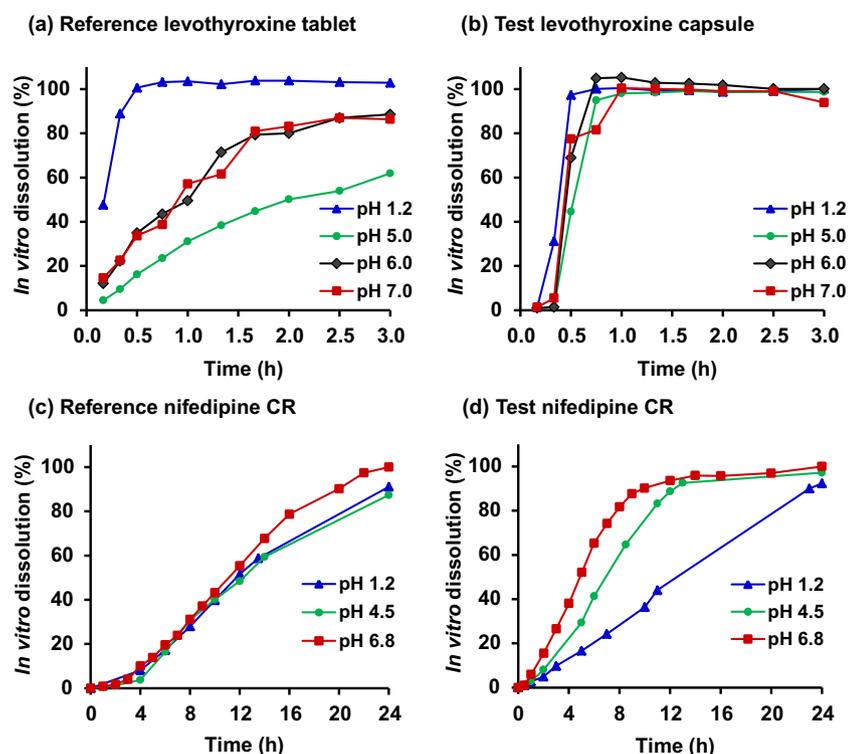


Fig. 2. *In vitro* dissolution profiles of the reference levothyroxine tablet (a) and capsule (b), and the reference (c) and test (d) nifedipine CR formulations extracted from the literature (Garbacz et al., 2009; Pabla et al., 2009).

using the Advanced Dissolution, Absorption and Metabolism (ADAM) model to model drug dissolution and absorption. The ADAM model within the Simcyp Simulator has already been described in detail (Darwich et al., 2010; Jamei et al., 2009). The ADAM model provides a variety of options for the input of dissolution rate information such as separate dissolution profiles for gastric and average small intestine pH values (for both the fed and fasted states). The dissolution rate was defined as a Weibull function, as shown in Eq. 1 and 2:

$$\%F_{diss} = F_{max(\%)} \cdot \left[1 - e^{-\frac{(t-lag)^\beta}{\alpha}} \right] \text{ when } t > lag \quad (1)$$

$$\%F_{diss} = 0 \text{ when } t \leq lag \quad (2)$$

where, $\%F_{diss}$ is the fraction of drug dose dissolved at time t , $F_{max(\%)}$ is the maximum % fraction of drug dose dissolved; lag is the lag time before dissolution begins; α and β are the Weibull scale and shape factors for the rate of dissolution, respectively.

The levothyroxine compound file was developed using the ADAM module. Physicochemical parameters (molecular weight, $\log P_{o:w}$, acid/base status and pK_a) and fraction unbound in plasma were obtained from data in the literature and public databases (IBSA Institut Biochimique SA, 2012; Svanfelt et al., 2011) (Table 1). Blood/plasma ratio was assumed to be 0.55 from fraction unbound in plasma and hematocrit reference value of 45% as almost all levothyroxine binds to plasma proteins (IBSA Institut Biochimique SA, 2012). The simultaneous estimation of the effective permeability in humans ($P_{eff,man}$), volume of distribution at steady state (V_{ss}), single adjusting compartment (SAC) parameters, and *in vivo* intravenous clearance (CL_{iv}) was carried out using the Parameter Estimation module in order to fit a minimal PBPK model to the observed data (Hays, 1991; Walter-Sack et al., 2004) (Table 1). The parameter values were estimated from simultaneous fitting with observed baseline-adjusted serum concentration-time profiles of levothyroxine in healthy volunteers after the intravenous administration ($n = 1$) and the oral administration of solution ($n = 24$) (Hays, 1991; Walter-Sack et al., 2004). The estimation was weighted by the number of individuals in each reported study. The performance for estimated parameters was verified by simulation

Table 1
Parameter values used for the levothyroxine simulations.

Parameters	Value	Reference/Comments
Molecular weight (g/mol)	776.9	PubChem
$\log P$	4.12	PubChem
Compound type	Ampholyte	–
pK_a	2.2, 10.1	(Svanfelt et al., 2011)
Fraction unbound in plasma	0.0004	(IBSA Institut Biochimique SA, 2012)
Blood/plasma ratio	0.55	Assumed from fraction unbound in plasma
Absorption Model	ADAM	
Fraction of drug unbound in enterocyte	1	Simcyp default
$P_{eff,man}$ ($\times 10^{-4}$ cm/s)	4.44	PE
Dissolution profile	See Table S1	Assumed by IVIVRs (See main text)
Distribution Model	Minimal PBPK	
V_{ss} (L/kg)	0.084	PE
SAC, K_{in} (1/h)	0.42	PE
SAC, K_{out} (1/h)	0.22	PE
V_{sac} (L/kg)	0.000015	PE
Elimination CL_{iv} (L/h)	0.13	PE

PE, parameter estimation using simultaneous fitting with observed data from the intravenous administration ($n = 1$) and the oral administration of solution ($n = 24$); ADAM model, Advanced Dissolution, Absorption and Metabolism model; IVIVR, *in vitro-in vivo* relationship: Minimal PBPK model, Minimal Physiologically-based Pharmacokinetic model; SAC, single adjusting compartment.

of 10 trial for 10 subjects ($n = 100$; Fig. S1). Levothyroxine concentration was predicted as baseline-adjusted levothyroxine because it is difficult to differentiate between exogenous levothyroxine and secreted endogenous thyroxine.

The pre-validated nifedipine compound file supplied in the Simcyp compound library was further developed to use the ADAM module for absorption modelling (Table 2). The $P_{eff,man}$ of nifedipine was estimated using *in vitro* MDCK II permeability data (Polli et al., 2001). The same

Table 2
Parameter values used for the nifedipine simulations.

Parameters	Value	Reference/Comments
Molecular weight (g/mol)	346.3	Simcyp V16
log P	2.69	Simcyp V16
Compound type	Monoprotic base	Simcyp V16
pK _a	2.82	Simcyp V16
Blood/plasma ratio	0.685	Simcyp V16
Fraction unbound in plasma	0.039	Simcyp V16
Absorption		
Model	ADAM	
Fraction of drug unbound in enterocyte	1	Simcyp V16
MDCK II permeability ($\times 10^{-6}$ cm/s)	61	(Polli et al., 2001)
Predicted P _{eff,man} ($\times 10^{-4}$ cm/s)	10.5	Predicted using MDCK II P _{app} -P _{eff} correlation model in ADAM
P _{eff,man} in colon ($\times 10^{-4}$ cm/s)	0.17	Assumed by sensitivity analysis
Dissolution profile	See Table S2	Assumed by IVIVCs (See main text)
Distribution		
Model	Minimal PBPK	
V _{ss} (L/kg)	0.57	Simcyp V16
Elimination		
rCYP3A4 V _{max} (pmol/min/pmol)	22	Simcyp V16
rCYP3A4 K _m (μ M)	10.95	Simcyp V16
rCYP3A5 V _{max} (pmol/min/pmol)	3.5	Simcyp V16
rCYP3A5 K _m (μ M)	31.9	Simcyp V16
Renal clearance (L/h)	Negligible	Simcyp V16

ADAM model, Advanced Dissolution, Absorption and Metabolism model; IVIVC, *in vitro-in vivo* correlation; Minimal PBPK model, Minimal Physiologically-based Pharmacokinetic model.

P_{eff,man} value was assumed throughout the seven small intestine segments from duodenum to ileum according to the default approach in the Simcyp Simulator. The P_{eff,man} value in colon was optimized using sensitivity analysis, because absorption of nifedipine in colon should be considered for CR formulation though the absorption was less rapid from the colon than from the upper part of the gut (Bode et al., 1996).

2.3. IVIVC modelling of dissolution profiles

The establishment of IVIVCs was performed using two-stage approach of IVIVC module within the Simcyp Simulator (Mistry et al., 2016). The current version of IVIVC module is available for adult humans only and uses only fasted state parameters. In the first step, the *in vivo* dissolution profiles were deconvolved from the observed plasma concentration profiles of the reference and test formulations. The deconvolved *in vivo* dissolution profiles were described using a Weibull function. In the second step, the deconvolved *in vivo* dissolution profiles from the first step were compared to the *in vitro* dissolution profiles in various pH buffers. The IVIVCs between the deconvolved *in vivo* dissolution profile and the *in vitro* dissolution profile were established in the condition with the slope of the regression line most closely aligned with a value of 1.0. If the slope of the regression line closely aligns with the identity line, the IVIVC provides higher confidence. If the IVIVC was not established well in above condition, *in vitro-in vivo* relationship (IVIVR) was explored by comparing time-dependent profiles between the *in vivo* dissolution and the *in vitro* dissolution in various pH buffers. When the deconvolved *in vivo* dissolution is considerably slower than *in vitro* dissolution at appropriate pH, it suggests that the dissolution/release is not the rate-limiting step of absorption. The IVIVR such as separate dissolution profiles for gastric and intestinal pH was established in the condition with the time-dependent profiles suggesting that the *in vivo* dissolution at the early time points would be over- or under-estimated by the dissolution at gastric pH. Once the IVIVC/Rs were established, the *in vitro* dissolution profiles and the deconvolved *in vivo* dissolution profiles were used for PBPK modelling (Table S1 and S2). *In vitro* dissolution profiles were fitted to a Weibull function using Matlab R2014a (Mathworks, Natick, MA, USA). The Weibull function values derived from the deconvolved *in vivo* dissolution profiles during

exploring IVIVC/R was used for PBPK modelling when the fitting of the *in vitro* dissolution profiles resulted in the α value exceeding 100 which could not be entered within the current version of Simcyp Simulator (Mistry et al., 2016) (Table S2).

2.4. Trial design

Virtual bioequivalence studies for levothyroxine were simulated using “healthy volunteers” within Simcyp population library. Trial design adapted to number of subjects, age, and proportion of females in the reported clinical study under fasted state (Colucci et al., 2011; Seng et al., 2015) (Table S3). The reported clinical study for levothyroxine under intravenous administration of esomeprazole enrolled subjects having a mean gastric pH ≥ 5 during the 2 h after the end of esomeprazole infusion over 30 min for a maximum dose of 80 mg (Seng et al., 2015). Levothyroxine formulations were administered to the subjects under fasting conditions with prior intravenous infusion of esomeprazole maximum 80 mg over 30 min. The intragastric pH was approximately 6.5 within the first 5 h following the start of the esomeprazole infusion (Seng et al., 2015). Virtual healthy volunteers under the intravenous administration of esomeprazole were designed by selecting achlorhydria frequency of 100% in default population. The oral dose of levothyroxine sodium was set to 0.6 mg with 250 mL water. The virtual bioequivalence studies were simulated 10 times.

Virtual bioequivalence studies for nifedipine CR were simulated using “healthy volunteers” and “Japanese” within Simcyp population library. Trial design for healthy volunteers adapted to number of subjects, age, and proportion of females in the reported clinical study under fasted/fed state (Schug et al., 2002b) (Table S4). Virtual Japanese achlorhydric elderly was designed by selecting age range of 60–70 years and achlorhydria frequency of 100% in default Japanese population. Trial design for Japanese achlorhydric elderly adapted to number of subjects and proportion of females for healthy volunteers (Table S4). The oral dose of nifedipine was set to 60 mg with 150 mL water. When the exposure parameters of nifedipine at steady state were investigated, the subjects received 60 mg once daily for 7 days. The virtual bioequivalence studies were simulated 10 times.

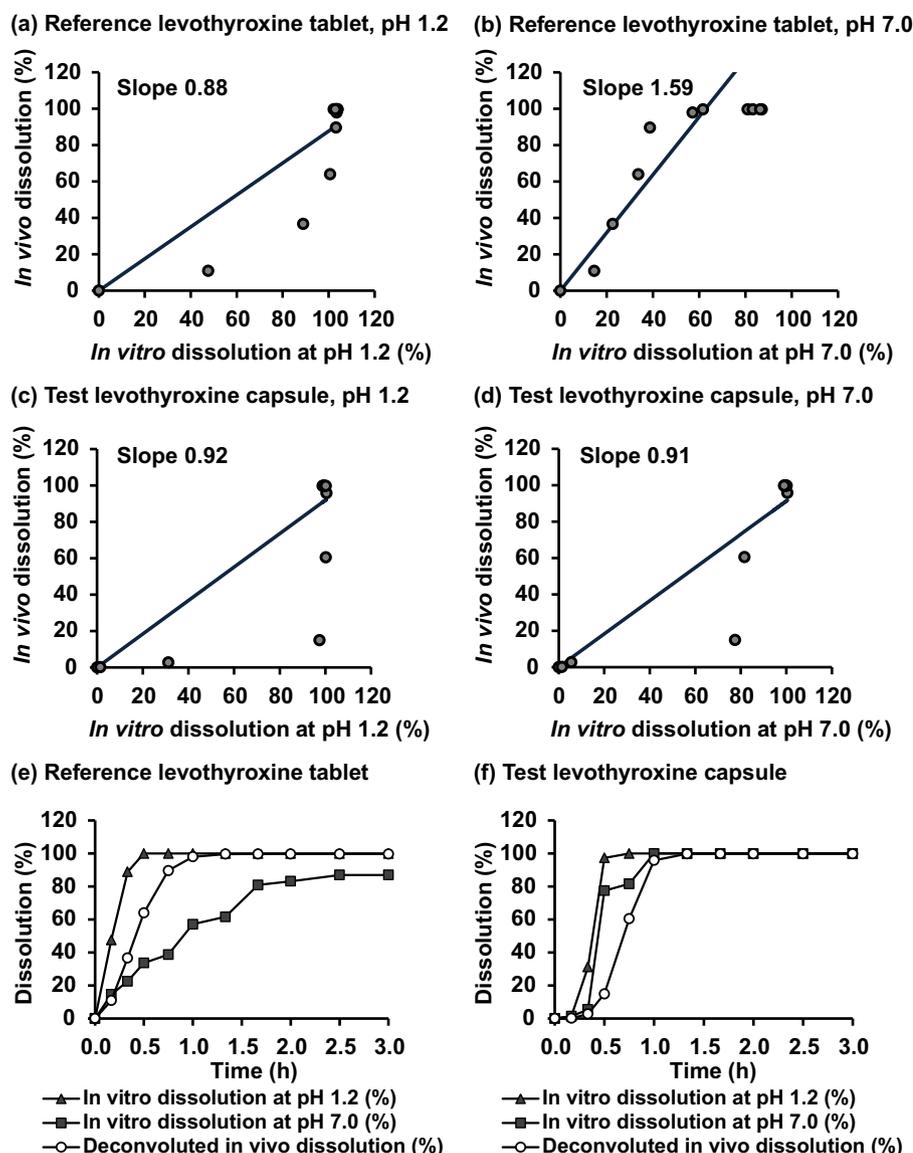


Fig. 3. *In vitro-in vivo* correlation between *in vitro* dissolution at pH 1.2 or 7.0, and deconvoluted *in vivo* dissolution for dissolution profiles of the reference tablet (a and b) and test capsule (c and d). Time-dependent profiles of *in vitro* dissolution at pH 1.2 and 7.0, and deconvoluted *in vivo* dissolution for the reference tablet (e) and test capsule (f).

2.5. Data analysis

Predicted C_{\max} (maximum serum concentration), T_{\max} (time to achieve C_{\max}), and AUC_{0-t} (area under the concentration curve from administration to 24 or 48 h) were compared to the observed values. Two formulations were deemed bioequivalent if the 90% confidence intervals (CI) of the geometric mean test/reference for C_{\max} and AUC ratios fall within the bioequivalence limits of 80–125% (Davitt et al., 2009). If the geometric mean test/reference for C_{\max} and AUC ratios has the potential to be influenced by different gastric dissolution, it was calculated by combining the simulated results derived from different *in vitro* dissolution profiles. Gastric pH after the intravenous administration of esomeprazole was generated randomly using R statistical software (version 3.3.2). The random generation was performed using the truncated normal distribution with reported mean and standard deviation (6.4 ± 0.9), and truncated on the interval (5.0–7.4) (Seng et al., 2015). The *in vitro* dissolution profiles at pH 5.0, 6.0, and 7.0 as gastric pH after the intravenous administration of esomeprazole were assigned to simulated subjects according to the truncated normal distribution.

3. Results

3.1. Virtual bioequivalence studies between levothyroxine tablet and capsule

The deconvoluted *in vivo* dissolution profiles for the reference tablet and the test capsule of levothyroxine were compared to the *in vitro* dissolution profiles in buffers of pH 1.2 and 7.0 (Fig. 3a–d). *In vivo* dissolution at the early time points for the reference levothyroxine tablet was under-estimated by *in vitro* dissolution at pH 7.0 (Fig. 3b). The comparison of time-dependent profiles between the *in vivo* and *in vitro* dissolution showed that the *in vivo* dissolution for the reference levothyroxine tablet was faster than *in vitro* dissolution at pH 7.0 but was slower than *in vitro* dissolution at pH 1.2, suggesting the IVIVR contributing to the *in vitro* dissolution profiles at pH 1.2 and 7.0 as gastric and intestinal dissolution profiles, respectively (Fig. 3e). *In vivo* dissolution for the test capsule did not correspond to *in vitro* dissolution profiles at pH 1.2 and pH 7.0 (Fig. 3c and d). The slower profiles of the *in vivo* dissolution than *in vitro* dissolution for the test capsule showed that release of dissolved levothyroxine is not rate-limiting step of absorption, suggesting that the *in vitro* dissolution profile related to *in vivo*

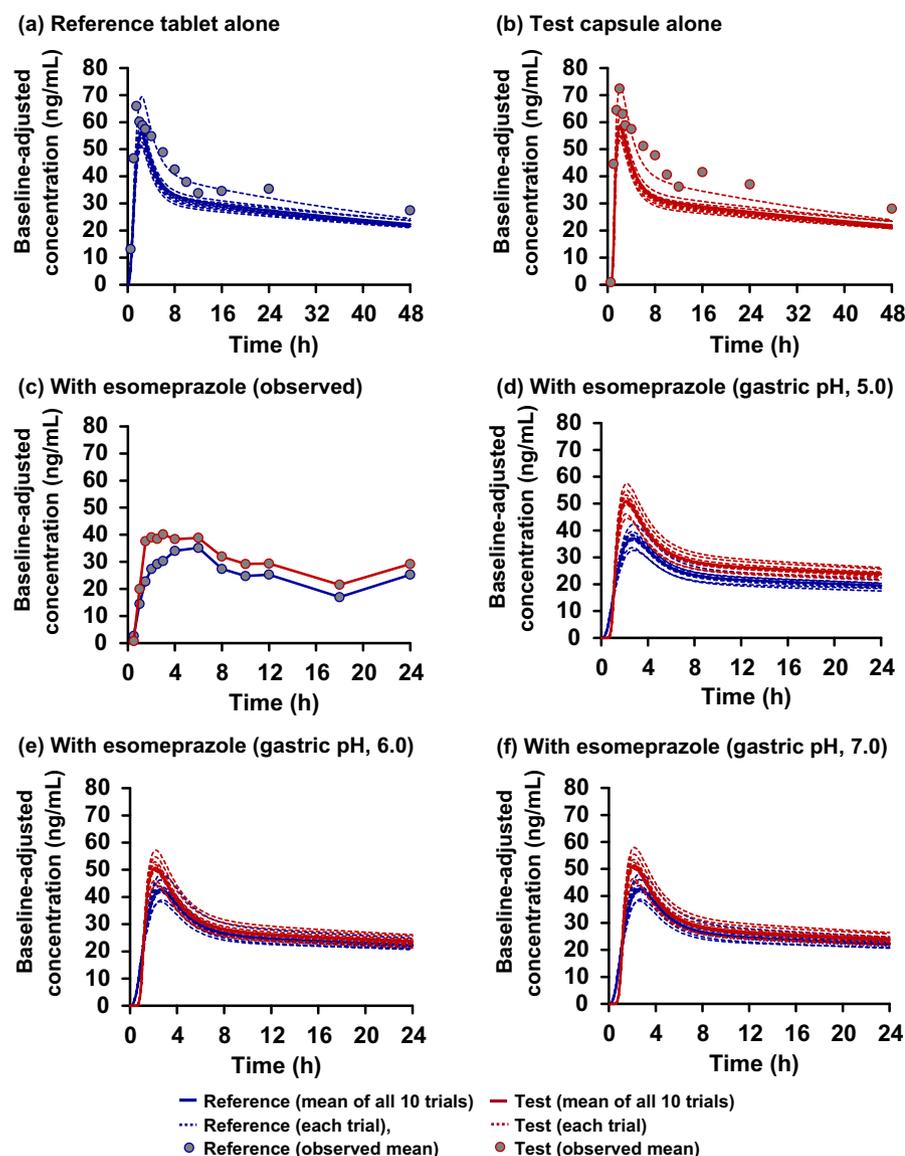


Fig. 4. Simulated and observed plasma levothyroxine concentration profiles for the reference tablet (a) and test capsule (b) of levothyroxine in healthy volunteers. Observed plasma levothyroxine concentration profiles for the reference and test formulations in healthy volunteers after the intravenous administration of esomeprazole (c) (Seng et al., 2015). Simulated plasma levothyroxine concentration profiles for the reference and test formulations in virtual healthy volunteers using gastric pH 5.0 (d), pH 6.0 (e), and pH 7.0 (f). The plasma levothyroxine concentrations were baseline-adjusted.

release profile for capsule formulation (Fig. 3f).

The predicted baseline-adjusted plasma levothyroxine profiles in healthy volunteers were successfully recovered for the reference and test formulations of levothyroxine using a PBPK model with separate *in vitro* dissolution profiles for stomach and intestine (pH 1.2 and 7.0, respectively; Table S1), producing results consistent with reported clinical data (Colucci et al., 2011) (Fig. 4a and b). The predicted C_{max} , T_{max} , and AUC were within 2-fold of the observed values for the reference and test formulation in healthy volunteers under fasted state (Table S5). The geometric mean test/reference ratios for C_{max} and AUC were 1.04 (90%CI, 0.99–1.10) and 0.97 (90%CI, 0.93–1.02) in healthy volunteers, respectively (Fig. 5 and Table S5). Subsequently, the model was applied to virtual achlorhydric healthy volunteer population where gastric pH mimicked that observed following intravenous administration of esomeprazole. Observed pharmacokinetic profiles of baseline-adjusted plasma levothyroxine in healthy volunteers after the intravenous administration of esomeprazole were shown in Fig. 4c. Pharmacokinetic profiles of baseline-adjusted plasma levothyroxine in virtual achlorhydric healthy volunteers were simulated using the *in vitro* dissolution profiles at gastric pH 5.0, 6.0, or 7.0 and intestinal pH 7.0 (Fig. 4d–f). The geometric mean test/reference ratios for C_{max} and AUC differed among gastric pH 5.0, 6.0, and 7.0: the difference in exposure between the reference and test formulations was more

pronounced in simulation using the *in vitro* dissolution profiles at gastric pH 5.0 (Fig. 5 and Table S5). The simulated results derived from different *in vitro* dissolution profiles were combined according to distribution derived from the reported gastric pH under the intravenous administration of esomeprazole (Fig. S2). The combined geometric mean test/reference ratios for C_{max} and AUC were 1.21 (90%CI, 1.13–1.29) and 1.09 (90%CI, 1.02–1.17), respectively (Fig. 5 and Table S5).

3.2. Virtual bioequivalence studies for nifedipine CR

The IVIVC produced a good correlation between *in vitro* and *in vivo* dissolution for the reference formulation without pH-dependency (Fig. 6a). *In vivo* dissolution for the test formulation under fasted and fed state corresponded to *in vitro* dissolution at pH 1.2 and pH 4.5, respectively (Fig. 6b, Fig. 6c).

The predicted plasma nifedipine profiles in healthy volunteers under fasted state were successfully recovered for the reference and test formulations of nifedipine CR using a PBPK model with deconvolved *in vivo* dissolution profiles, producing results consistent with reported clinical data (Schug et al., 2002b) (Fig. 7a and b). The predicted C_{max} , T_{max} , and AUC were within 2-fold of the observed values for the reference and test formulation in healthy volunteers under fasted state

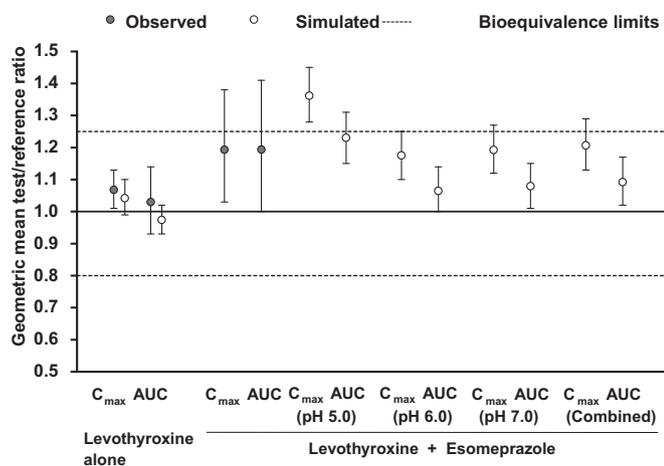


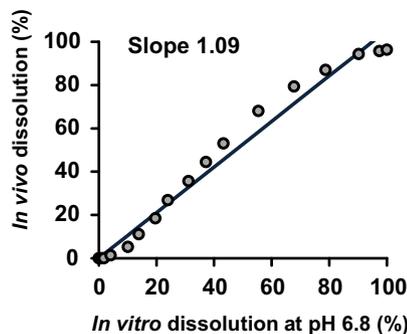
Fig. 5. The geometric mean ratios in drug exposure parameters for the reference tablet and test capsule of levothyroxine. Simulations of drug exposure parameters under intravenous esomeprazole were performed using gastric pH 5.0, 6.0, and 7.0. The results from simulations using each gastric pH were combined based on distribution of gastric pH generated from the reported value after the intravenous administration of esomeprazole. The observed and simulated ratios were shown as geometric mean and 90% confidence intervals. C_{max} , maximum serum concentration; AUC, area under the concentration curve from administration to 24 or 48 h; Bioequivalence limits, 0.8–1.25.

(Table S6). The geometric mean test/reference ratios for C_{max} , AUC_{0-24} , and AUC_{0-48} were 0.98 (90%CI, 0.89–1.07), 0.92 (90%CI, 0.84–1.01) and 0.91 (90%CI, 0.83–1.00) in healthy volunteers under fasted state, respectively (Table S6). The PBPK model also reproduced formulation-specific food effects using the *in vivo* dissolution profile for the reference formulation and *in vitro* dissolution at pH 4.5 for the test formulation: the geometric mean test/reference ratios for C_{max} , AUC_{0-24} , and AUC_{0-48} were 2.02 (90%CI, 1.83–2.23), 1.50 (90%CI, 1.38–1.64) and 1.38 (90%CI, 1.26–1.52), respectively (Fig. 7c, Table S6). Subsequently, the model was applied to Japanese achlorhydric elderly using the *in vivo* dissolution profile for the reference formulation and *in vitro* dissolution at pH 6.8 for the test formulation (Fig. 7d). The predicted plasma nifedipine concentration profiles in Japanese achlorhydric elderly subjects showed difference in exposure between the reference and test formulations: the geometric mean test/reference ratios for C_{max} , AUC_{0-24} , and AUC_{0-48} were 3.08 (90%CI, 2.81–3.38), 1.87 (90%CI, 1.71–2.05) and 1.57 (90%CI, 1.43–1.74), respectively (Fig. 8, Table S6). The exposure parameters under steady state were also examined after the repeated administration of nifedipine CR once daily for 7 days. The geometric mean test/reference ratios for C_{max} and AUC_{0-24} after last dose were 2.82 (90%CI, 2.57–3.08) and 1.55 (90%CI, 1.41–1.70), respectively (Table S7).

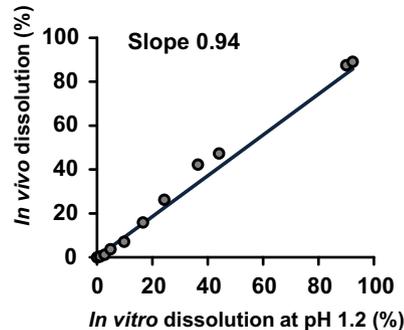
4. Discussion

Appropriate implementation of a virtual bioequivalence study using a PBPK model may postulate *in vivo* consequences of any differences between formulations in specific conditions such as achlorhydria. Incorporation of the ADAM model using different dissolution profiles into a PBPK disposition model allowed simulation of pharmacokinetic profiles for drug formulations where pH-sensitivity for *in vitro* dissolution differs. Since *in vitro* dissolution profile does not necessarily correspond to *in vivo* dissolution profile under the influences of various physiological conditions such as gastric emptying, gastrointestinal pH and fluid dynamics, IVIVC modelling is essential to evaluate and interpret the similarity or difference between *in vitro* and *in vivo* dissolution profiles (Sjögren et al., 2014). The pH-dependent difference of *in vitro* dissolution profile would be applicable in PBPK modelling when IVIVC/R was established between *in vitro* dissolution profile at appropriate pH and deconvolved *in vivo* dissolution profile. The PBPK model

(a) Reference nifedipine CR, fasted state



(b) Test nifedipine CR, fasted state



(c) Test nifedipine CR, fed state

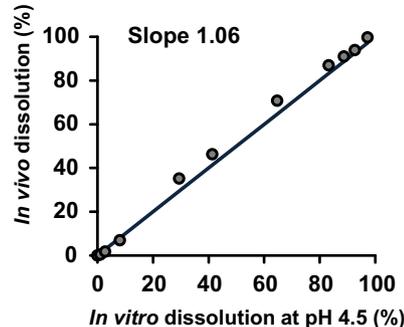


Fig. 6. *In vitro-in vivo* correlation for dissolution profiles of the reference nifedipine CR formulation under fasted state (a) and test nifedipine CR formulation under fasted (b) and fed state (c).

using dissolution profiles can be used for virtual bioequivalence study in specific patient populations after model verification using clinical data from bioequivalence study in healthy volunteers.

The levothyroxine PBPK model for the virtual bioequivalence study was developed through the use of physicochemical properties and pharmacokinetic observations from the administration of intravenous and oral solution (Fig. S1). The model did not produce IVIVC between deconvolved *in vivo* dissolution and *in vitro* dissolution at both pH 1.2 and 7.0 (Fig. 3). Time-dependent profiles between the *in vivo* and *in vitro* dissolution suggested IVIVRs for dissolution from the reference and test levothyroxine formulations (Fig. 3e and f). The time-dependent profiles of dissolution for the reference tablet showed that the *in vivo* dissolution was enhanced at the early time points involved with dissolution at stomach. This finding implied that the higher solubility at gastric pH resulted in the faster *in vivo* dissolution than *in vitro* dissolution at intestinal pH. Therefore, an IVIVR using separate *in vitro* dissolution profiles for gastric and intestinal pH could be incorporated into the levothyroxine PBPK model. The deconvolved *in vivo* dissolution for the test capsule was slower than *in vitro* dissolution without pH-sensitivity. This result is supported by characteristics of the test formulation that rapidly releases levothyroxine dissolved in glycerin

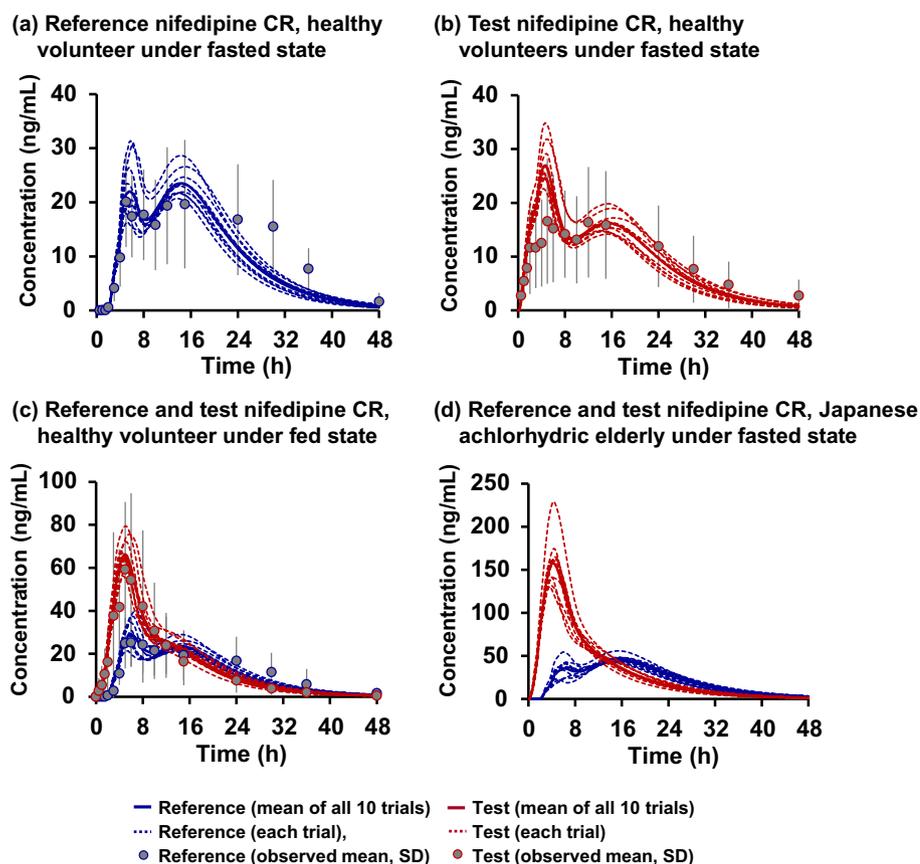


Fig. 7. Simulated and observed plasma nifedipine concentration profiles for the reference (a) and test (b) nifedipine CR formulations in healthy volunteers under fasted state. Simulated and observed plasma nifedipine concentration profiles for the reference and test formulations in healthy volunteers under fed state (c). Simulated plasma nifedipine concentration profiles for the reference and test formulations in Japanese achlorhydric elderly (d).

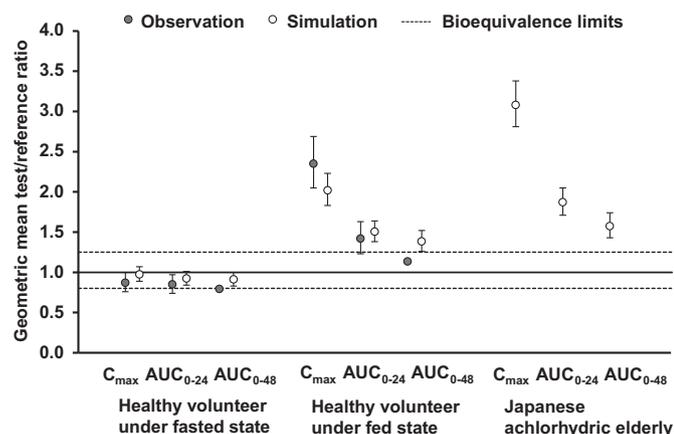


Fig. 8. The geometric mean ratios in drug exposure parameters for the reference and test nifedipine CR formulations in healthy volunteers at fasted and fed state, and Japanese achlorhydric elderly. The observed and simulated ratios were shown as geometric mean and 90% confidence intervals (CI), except for observed AUC_{0-48} ratio which 90% CIs were not reported in literature. C_{max} , maximum serum concentration; AUC, area under the concentration curve from administration to 24 or 48 h; Bioequivalence limits, 0.8–1.25.

(Fiorini et al., 2016). The *in vitro* dissolution profile for the test levothyroxine capsule could be used as release profile because release/dissolution was not the rate-limiting step of absorption. The PBPK model using the *in vitro* dissolution profile reproduced the pharmacokinetic profiles of levothyroxine for the reference tablet and test capsule (Fig. 4a and b). Thus, the PBPK model using *in vitro* dissolution profiles was applicable in virtual bioequivalence study to assess the effect of proton pump inhibitor on bioequivalence between levothyroxine formulations. The geometric mean test/reference for C_{max} and AUC ratios were calculated by combining the simulated results derived from different *in vitro* dissolution profiles, because the reference levothyroxine

tablet shows difference in the *in vitro* dissolution profile among pH 5.0, 6.0, and 7.0. The virtual bioequivalence study successfully reproduced the geometric mean test/reference for C_{max} and AUC ratios from results observed in the *in vivo* bioequivalence study (Fig. 5). This virtual bioequivalence study could raise the possibility that bioequivalence outcome for levothyroxine formulations was not similar between healthy volunteers and achlorhydric subjects induced by proton pump inhibitor. However, simulation in the geometric mean test/reference for C_{max} and AUC ratios showed narrower range of the 90% CIs than those in observation. This difference may be caused by the lack of considering inter-occasion variability in absorption because the administration of proton pump inhibitor is possible to induce changes in physiological factors such as gastric emptying rate and small intestinal transit time (Rasmussen et al., 1999). Development of appropriate population approaches handling inter-occasion variability is important for filling gaps in the accurate prediction of variability in *in vivo* bioequivalence between formulations.

The deconvolved *in vivo* dissolution profiles obtained in the first step of IVIVC modelling were employed for the construction of PBPK model to simulate the plasma nifedipine profiles for the reference and test formulations in healthy volunteers at fasted state (Fig. 7a and b). The PBPK model using the deconvolved *in vivo* dissolution profiles for the reference formulation without pH-dependency reproduced the observed plasma nifedipine profile in healthy volunteers at not only fasted but also fed state (Fig. 7a–c). In contrast, the test formulation shows the pH-sensitive *in vitro* dissolution profiles (Fig. 2). The *in vivo* dissolution profile deconvolved from pharmacokinetic profile for the test formulation in healthy volunteers under fasted state corresponded to the *in vitro* dissolution profile at pH 1.2 but did not to that at pH 6.8 (Fig. 6b). The result suggested that acidity in gastric fluids where the test formulation first contacts could determine the *in vivo* dissolution profile. This mechanism is supported by characteristics of test formulation that the immediate interaction between the polymeric substances contained

in the test formulation and water/biological fluids causes the hydration and the distension of the polymeric chains, and control the subsequent dissolution of nifedipine (So.Se.PHARM S.r.l., 2016). The deconvolved *in vivo* dissolution profile for the test formulation under fed state also corresponded to the *in vitro* dissolution profile at pH 4.5 (Fig. 6c) where is close to gastric acidity during the meal, pH 4.9 (Russell et al., 1993), although the parameters (e.g., gastric emptying time and bile salt concentration) other than gastric pH also change under fed conditions. The PBPK model using the *in vitro* dissolution profile at pH 4.5 reproduced formulation-specific food effect for the test formulation (Fig. 7c). These results based on PBPK and IVIVC modelling suggested that a PBPK model using *in vitro* dissolution profile at pH 6.8 for the test formulation was applicable in virtual bioequivalence study to assess the effect of achlorhydria on bioequivalence of nifedipine CR formulations. The virtual bioequivalence study through PBPK and IVIVC modelling revealed that Japanese elderly patients may have altered exposure outside the bioequivalence limits (80–125%) due to higher frequency of achlorhydria (Fig. 8, Table S6 and S7).

The PBPK model for nifedipine CR formulations produced a second peak in plasma nifedipine profiles (Fig. 7a and b) similar to reported clinical data (Schug et al., 2002b). This second peak may attribute to absorption of nifedipine in colon, since it was highly sensitive to the $P_{\text{eff,man}}$ value in colon when was optimized using sensitivity analysis. The predicted plasma nifedipine profile for the reference formulation was underestimated at 24 h or later after administration compared to observed profile (Fig. 7a). The previous study has reported that an ADAM-PBPK model in conjunction with diffusion layer model (DLM) successfully predicted nifedipine pharmacokinetics at 24 h or later for the reference nifedipine CR formulations despite nifedipine release mostly completed at 24 h (Patel et al., 2014). The ADAM model with DLM assuming that the rate limiting step is diffusion across a boundary layer around the solid drug particle of constant thickness handles supersaturation and precipitation (Wang and Flanagan, 1999). The DLM was not used in the modelling work for this study. The assumptions may improve the prediction of pharmacokinetic profile for the reference formulation, especially pharmacokinetic profile at 24 h or later. However, the purpose of this study is to assess virtual bioequivalence using a PBPK model and *in vitro* dissolution profile establishing IVIVC.

Bioequivalence guidelines have more similarities than differences in the approaches among the regulatory authorities in Australia, Brazil, Canada, China, Chinese Taipei, the European Medicines Association, Japan, Mexico, Singapore, South Korea, Switzerland, the USA, and the World Health Organization (Davitt et al., 2013). The recommended bioequivalence study design is a randomized, single-dose, two-way crossover in healthy normal subjects. The regulatory authority in Japan recommends *in vivo* bioequivalence study in subjects with low gastric acidity in cases where the test and reference products show a significant difference in *in vitro* dissolution at around pH 6.8, or between pH 3.0–6.8 for basic drugs (MHLW, 2012). The other regulatory authorities have no recommendation in these cases despite the fact that bioequivalence cannot be necessarily assumed the same in patients and healthy volunteers population. However, we faces situation where proton pump inhibitors which induce low gastric acidity are extensively used around world, and the prevalence of proton pump inhibitors use increases with age (Hassing et al., 2016; Lazarus et al., 2016; Lødrup et al., 2014; Nishtala and Soo, 2013; Patterson et al., 2013). Therefore, the effect of low gastric acidity on bioequivalence outcome should be investigated if the test and reference products show a significant difference in *in vitro* dissolution at neutral pH. An *in vivo* bioequivalence study in subjects with low gastric acidity could be conducted with difficulties such as recruitment of achlorhydric subjects whose prevalence is typically less in healthy volunteers than in Japanese elderly and temporary induction of achlorhydria by gastric acid reducers in healthy subjects. Under certain conditions, virtual bioequivalence study through PBPK framework including IVIVC modelling may be proposed as an alternative means of *in vivo* bioequivalence study in subjects with

low gastric acidity.

The present virtual bioequivalence studies for levothyroxine and nifedipine CR through the PBPK models indicated the possibilities that bioequivalence was not established between the reference and test formulations in achlorhydric subjects. However, the disparity in bioequivalence outcome between healthy volunteers and achlorhydric subjects was less pronounced for levothyroxine formulations as compared to nifedipine CR. This attributes to difference in pharmaceutical properties such as release rate (e.g. immediate release and extended release) and release system (e.g. OROS and hydrophilic matrix). The formulation-specific bioequivalence outcome in achlorhydric subjects would be also influenced by other drug properties such as low and high permeability, low and high metabolism, and involvement with pH-sensitive uptake transporter. The peptide transporter PEPT1 mediates active absorption of peptidomimetic drugs with proton gradient and luminal pH providing the driving force, and the PEPT1 substrates exhibit different pH-sensitive transport profiles (Brodin et al., 2002; Nozawa et al., 2003; Wenzel et al., 1996). Future virtual bioequivalence studies for achlorhydric subjects may be interested in drugs of various pharmaceutical classes and pH-sensitive uptake transporter substrates, and may offer the possibility to make a kind of classification about the need or not for specific bioequivalence study.

5. Conclusions

Virtual bioequivalence studies through the PBPK models successfully predicted the bioequivalence outcomes for levothyroxine and nifedipine CR formulations in healthy volunteers. However, it suggested that Japanese elderly and patients receiving proton pump inhibitor may have altered exposure outside the bioequivalence limits (80–125%) due to higher frequency of achlorhydria. The reverse could be true if the formulations behaved differently in acidic media but similar in basic environment. Virtual bioequivalence studies through the PBPK models can highlight the need for conduct of specific studies in elderly Japanese populations where there are discrepancies in pH-sensitivity of dissolution between the test and reference formulations. Further development of PBPK modelling, such as considering inter-occasion variability, may allow providing the accurate prediction of variability in *in vivo* bioequivalence between formulations.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at <http://dx.doi.org/10.1016/j.ejps.2017.07.035>.

References

- Bode, H., Brendel, E., Ahr, G., Fuhr, U., Harder, S., Staib, A.H., 1996. Investigation of nifedipine absorption in different regions of the human gastrointestinal (GI) tract after simultaneous administration of 13C- and 12C-nifedipine. *Eur. J. Clin. Pharmacol.* 50, 195–201.
- Brodin, B., Nielsen, C.U., Steffansen, B., Frøkjær, S., 2002. Transport of peptidomimetic drugs by the intestinal Di/tri-peptide transporter, PepT1. *Pharmacol. Toxicol.* 90, 285–296.
- Colucci, P., D'Angelo, P., Mautone, G., Scarsi, C., Ducharme, M.P., 2011. Pharmacokinetic equivalence of a levothyroxine sodium soft capsule manufactured using the new food and drug administration potency guidelines in healthy volunteers under fasting conditions. *Ther. Drug Monit.* 33, 355–361.

- Cristofoletti, R., Patel, N., Dressman, J.B., 2017. Assessment of bioequivalence of weak base formulations under various dosing conditions using physiologically based pharmacokinetic simulations in virtual populations. Case Examples: Ketoconazole and Posaconazole. *J. Pharm. Sci.* 106, 560–569.
- Darwich, A.S., Neuhoﬀ, S., Jamei, M., Rostami-Hodjegan, A., 2010. Interplay of metabolism and transport in determining oral drug absorption and gut wall metabolism: a simulation assessment using the “Advanced Dissolution, Absorption, Metabolism (ADAM)” model. *Curr. Drug Metab.* 11, 716–729.
- Davit, B.M., Nwakama, P.E., Buehler, G.J., Conner, D.P., Haidar, S.H., Patel, D.T., Yang, Y., Yu, L.X., Woodcock, J., 2009. Comparing generic and innovator drugs: a review of 12 years of bioequivalence data from the United States Food and Drug Administration. *Ann. Pharmacother.* 43, 1583–1597.
- Davit, B., Braddy, A.C., Conner, D.P., Yu, L.X., 2013. International guidelines for bioequivalence of systemically available orally administered generic drug products: a survey of similarities and differences. *AAPS J.* 15, 974–990.
- Fiorini, G., Ribichini, D., Pasquali, R., Vaira, D., 2016. In vivo dissolution of levothyroxine soft gel capsules. *Intern. Emerg. Med.* 11, 1151–1152.
- Garbacz, G., Golke, B., Wedemeyer, R.S., Axell, M., Söderlind, E., Abrahamsson, B., Weitschies, W., 2009. Comparison of dissolution profiles obtained from nifedipine extended release once a day products using different dissolution test apparatuses. *Eur. J. Pharm. Sci.* 38, 147–155.
- Hassing, R.J., Verbon, A., de Visser, H., Hofman, A., Stricker, B.H., 2016. Proton pump inhibitors and gastroenteritis. *Eur. J. Epidemiol.* 31, 1057–1063.
- Hays, M.T., 1991. Localization of human thyroxine absorption. *Thyroid* 1, 241–248.
- IBSA Institut Biochimique SA, 2012. Prescribing information for TIROSINT®. (<http://www.tirosint.com/wp-content/uploads/2016/05/Tirosint-Promo-PI.pdf> (accessed: 31.10.16)).
- Jamei, M., Turner, D., Yang, J., Neuhoﬀ, S., Polak, S., Rostami-Hodjegan, A., Tucker, G., 2009. Population-based mechanistic prediction of oral drug absorption. *AAPS J.* 11, 225–237.
- Klintermalm, G.B., 2011. Immunosuppression, generic drugs and the FDA. *Am. J. Transplant.* 11, 1765–1766.
- Lazarus, B., Chen, Y., Wilson, F.P., Sang, Y., Chang, A.R., Coresh, J., Grams, M.E., 2016. Proton pump inhibitor use and the risk of chronic kidney disease. *JAMA Intern. Med.* 176, 238–246.
- Lødrup, A., Reimer, C., Bytzer, P., 2014. Use of antacids, alginates and proton pump inhibitors: a survey of the general Danish population using an internet panel. *Scand. J. Gastroenterol.* 49, 1044–1050.
- MHLW (The Ministry of Health, Labour and Welfare), 2012. Guideline for Bioequivalence Studies of Generic Products. ([http://www.nihs.go.jp/drug/be-guide\(e\)/Generic/GL-E_120229_BE_rev140409.pdf](http://www.nihs.go.jp/drug/be-guide(e)/Generic/GL-E_120229_BE_rev140409.pdf) (accessed 31.10.16)).
- Mistry, B., Patel, N., Jamei, M., Rostami-Hodjegan, A., Martinez, M.N., 2016. Examining the use of a mechanistic model to generate an in vivo/in vitro correlation: journey through a thought process. *AAPS J.* 18, 1144–1158.
- Morihara, M., Aoyagi, N., Kaniwa, N., Kojima, S., Ogata, H., 2001. Assessment of gastric acidity of Japanese subjects over the last 15 years. *Biol. Pharm. Bull.* 24, 313–315.
- Nishtala, P.S., Soo, L., 2013. Proton pump inhibitors utilisation in older people in New Zealand from 2005 to 2013. *Intern. Med. J.* 45, 624–629.
- Nozawa, T., Toyobuku, H., Kobayashi, D., Kuruma, K., Tsuji, A., Tamai, I., 2003. Enhanced intestinal absorption of drugs by activation of peptide transporter PEPT1 using proton-releasing polymer. *J. Pharm. Sci.* 92, 2208–2216.
- Pabla, D., Akhlaghi, F., Zia, H., 2009. A comparative pH-dissolution profile study of selected commercial levothyroxine products using inductively coupled plasma mass spectrometry. *Eur. J. Pharm. Biopharm.* 72, 105–110.
- Patel, N., Polak, S., Jamei, M., Rostami-Hodjegan, A., Turner, D.B., 2014. Quantitative prediction of formulation-specific food effects and their population variability from in vitro data with the physiologically-based ADAM model: a case study using the BCS/BDDCS Class II drug nifedipine. *Eur. J. Pharm. Sci.* 57, 240–249.
- Patterson, Burdsall D., Flores, H.C., Krueger, J., Garretson, S., Gorbien, M.J., Iacch, A., Dobbs, V., Homa, T., 2013. Use of proton pump inhibitors with lack of diagnostic indications in 22 Midwestern US skilled nursing facilities. *J. Am. Med. Dir. Assoc.* 14, 429–432.
- Polli, J.W., Wring, S.A., Humphreys, J.E., Huang, L., Morgan, J.B., Webster, L.O., Serabjit-Singh, C.S., 2001. Rational use of in vitro P-glycoprotein assays in drug discovery. *J. Pharmacol. Exp. Ther.* 299, 620–628.
- Rasmussen, L., Oster-Jørgensen, E., Qvist, N., Pedersen, S.A., 1999. The effects of omeprazole on intragastric pH, intestinal motility, and gastric emptying rate. *Scand. J. Gastroenterol.* 34, 671–675.
- Russell, T.L., Berardi, R.R., Barnett, J.L., Dermentzoglou, L.C., Jarvenpaa, K.M., Schmalz, S.P., Dressman, J.B., 1993. Upper gastrointestinal pH in seventy-nine healthy, elderly, North American men and women. *Pharm. Res.* 10, 187–196.
- Schug, B.S., Brendel, E., Chantraine, E., Wolf, D., Martin, W., Schall, R., Blume, H.H., 2002a. The effect of food on the pharmacokinetics of nifedipine in two slow release formulations: pronounced lag-time after a high fat breakfast. *Br. J. Clin. Pharmacol.* 53, 582–588.
- Schug, B.S., Brendel, E., Wolf, D., Wonnemann, M., Wargenau, M., Blume, H.H., 2002b. Formulation-dependent food effects demonstrated for nifedipine modified-release preparations marketed in the European Union. *Eur. J. Pharm. Sci.* 15, 279–285.
- Seng, Yue C., Benvenega, S., Scarsi, C., Loprete, L., Ducharme, M.P., 2015. When bioequivalence in healthy volunteers may not translate to bioequivalence in patients: differential effects of increased gastric pH on the pharmacokinetics of levothyroxine capsules and tablets. *J. Pharm. Sci.* 18, 844–855.
- Sjögren, E., Abrahamsson, B., Augustijns, P., Becker, D., Bolger, M.B., Brewster, M., Brouwers, J., Flanagan, T., Harwood, M., Heinen, C., Holm, R., Juretschke, H.P., Kubbinga, M., Lindahl, A., Lukacova, V., Münster, U., Neuhoﬀ, S., Nguyen, M.A., Peer, Av, Reppas, C., Hodjegan, A.R., Tannergren, C., Weitschies, W., Wilson, C., Zane, P., Lennernäs, H., Langguth, P., 2014. In vivo methods for drug absorption - comparative physiologies, model selection, correlations with in vitro methods (IVIVC), and applications for formulation/API/excipient characterization including food effects. *Eur. J. Pharm. Sci.* 57, 99–151.
- So.Se.PHARM S.r.l., 2016. CORAL® Summary of Product Characteristics. (https://farmaci.agenziafarmaco.gov.it/aifa/servlet/PdfDownloadServlet?pdfFileName=footer_008007_024599_RCP.pdf&retry=0&sys=m0b113 (accessed 31.10.16)).
- Svanfelt, J., Eriksson, J., Kronberg, L., 2011. Photochemical transformation of the thyroid hormone levothyroxine in aqueous solution. *Environ. Sci. Pollut. Res. Int.* 18, 871–876.
- Walter-Sack, I., Clanget, C., Ding, R., Goeggelmann, C., Hinke, V., Lang, M., Pfeilschifter, J., Tayrouz, Y., Wegscheider, K., 2004. Assessment of levothyroxine sodium bioavailability: recommendations for an improved methodology based on the pooled analysis of eight identically designed trials with 396 drug exposures. *Clin. Pharmacokinet.* 43, 1037–1053.
- Wang, J., Flanagan, D.R., 1999. General solution for diffusion-controlled dissolution of spherical particles. 1. Theory. *J. Pharm. Sci.* 88, 731–738.
- Wenzel, U., Gebert, I., Weintraut, H., Weber, W.M., Clauss, W., Daniel, H., 1996. Transport characteristics of differently charged cephalosporin antibiotics in oocytes expressing the cloned intestinal peptide transporter PevT1 and in human intestinal Caco-2 cells. *J. Pharmacol. Exp. Ther.* 277, 831–839.
- Wonnemann, M., Schug, B., Anshütz, M., Brendel, E., De Nucci, G., Blume, H., 2008. Comparison of two marketed nifedipine modified-release formulations: an exploratory clinical food interaction study. *Clin. Ther.* 30, 48–58.