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Original Article

Development and validation of an *in vitro*–*in vivo* correlation (IVIVC) model for propranolol hydrochloride extended-release matrix formulations

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ABSTRACT

The objective of this study was to develop an *in vitro*–*in vivo* correlation (IVIVC) model for hydrophilic matrix extended-release (ER) propranolol dosage formulations. The *in vitro* release characteristics of the drug were determined using USP apparatus I at 100 rpm, in a medium of varying pH (from pH 1.2 to pH 6.8). *In vivo* plasma concentrations and pharmacokinetic parameters in male beagle dogs were obtained after administering oral, ER formulations and immediate-release (IR) commercial products. The similarity factor f_2 was used to compare the dissolution data. The IVIVC model was developed using pooled fraction dissolved and fraction absorbed of propranolol ER formulations, ER-F and ER-S, with different release rates. An additional formulation ER-V, with a different release rate of propranolol, was prepared for evaluating the external predictability. The results showed that the percentage prediction error (%PE) values of C_{max} and $AUC_{0-\infty}$ were 0.86% and 5.95%, respectively, for the external validation study. The observed low prediction errors for C_{max} and $AUC_{0-\infty}$ demonstrated that the propranolol IVIVC model was valid.

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1. Introduction

In vitro–*in vivo* correlation (IVIVC) plays a key role in the pharmaceutical development of dosage forms. IVIVC can serve as a surrogate for *in vivo* bioavailability and to support biowaivers. It also allows setting of the dissolution specification and methods [1,2]. In order to prove the validity of a new formulation, a bioequivalence study may be needed, taking a

considerable amount of time and money. Thus, the application of IVIVC attracts the attention of the pharmaceutical industry.

Development and validation are two critical stages in the evaluation of an IVIVC model. In the first stage, the development of a level A IVIVC model is usually estimated by a two-stage process [1]. At the first stage, the observed fraction of the drug absorbed is estimated using the numerical

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deconvolution method. The IVIVC model is developed by using the observed fraction of the drug absorbed and that of the drug dissolved. Based on the IVIVC model, the predicted fraction of the drug absorbed is calculated from the observed fraction of the drug dissolved. The predicted fraction of the drug absorbed is then convolved to the predicted plasma concentrations by using the convolution method. In the second stage, the predictability evaluation of the IVIVC model should focus on estimating the percent prediction error (%PE) between the observed and predicted plasma concentration profiles, such as the difference in pharmacokinetic parameters [C_{\max} and the area under the curve from time zero to infinity ($AUC_{0-\infty}$)]. The internal and/or external evaluation of the %PE may also be appropriate. The internal predictability is based on the initial data used to define the IVIVC model, and the external predictability is based on the additional data [1].

Propranolol is a non-selective beta adrenergic blocking agent and is widely used for the treatment of angina pectoris, hypertension, and many other cardiovascular disorders. After oral administration, propranolol is almost completely absorbed. However, the bioavailability of propranolol is extremely limited (30%), due to the hepatic first-pass effect, and its elimination half-life is also relatively short (approximately 2–6 hours) [3]. For hypertension treatment, the usual dose is 120–240 mg divided in 2–3 doses/day; the maximum daily dose is 640 mg. Therefore, propranolol was a good candidate for the preparation of the once-daily extended-release (ER) dosage formulation. Many IVIVC studies have been reported regarding controlled-release formulations [4–10], but there are none regarding propranolol matrix ER formulations. Thus, developing an IVIVC model of propranolol ER tablets is beneficial for obtaining biowaivers for scale-up and certain pre- or post-approval changes. The objective of this study was to develop an IVIVC model for propranolol ER dosage formulations. The validation of the internal and external predictabilities was completed for a wide range of formulations. In addition, IVIVC of the drug in the animal models provides the feasibility of the drug delivery system for a given drug candidate. The objective of this study was to use propranolol as a model drug, using hydroxypropyl methylcellulose (HPMC), Avicel, and lactose to develop formulations with different release rates, and also to set up the IVIVC animal model to evaluate the feasibility of the drug delivery system. Such an approach may also be applied to the development of other drug candidates in the future.

2. Methods

2.1. Materials and equipment

Propranolol hydrochloride and p-hydroxybenzoate-butyl ester were purchased from TCI Co. (Tokyo, Japan), hydroxypropyl methylcellulose (HPMC) was from Shin Etsu, (Tokyo, Japan), microcrystalline cellulose (Avicel) was from Asahi Co. (Tokyo, Japan), and lactose was from New Zealand Lactose Co (Hawera, New Zealand). All chemicals and solvents used were of analytical reagent grade.

Six Beagles dogs used in this study were supplied from the animal center of National Pingtung University of Science and

Technology (NPUST) (Pingtung, Taiwan). Each adult beagle dog weighed between 8 and 14 kg.

2.2. Formulations

ER tablets of propranolol hydrochloride formulated using HPMC, Avicel, and lactose for modifying the release rates have been discussed previously [11]. Two ER tablets were designed to release propranolol at two different rates referred to as: slow (ER-S) and fast (ER-F) for the development of the IVIVC model. The compositions of these ER tablets are shown in Table 1. For evaluating the external predictability of the IVIVC model, an additional formulation, ER-V, with a release rate between those of formulation ER-S and ER-F, was prepared; it underwent dissolution test and *in vivo* absorption studies.

2.3. Dissolution test

The release characteristics of the propranolol ER tablets were determined using USP apparatus I, the basket method. The rotation speed was set at 100 rpm. The propranolol tablets were placed in 900 mL of gastric fluid and maintained at 37°C. Samples (5 mL) were collected, each at an appropriate interval. After 1.5 hours, the pH of the dissolution medium was varied from 1.2 to 6.8 by adding 80 mL of concentrated phosphate buffer to simulate the intestinal fluid, and then the experiment was run for the specified time. The dissolution samples were collected at the following time intervals: 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, and 24 hours. The amount of drug released was analyzed by ultraviolet/visible spectrophotometry at 290 nm wavelength. At least six tablets of each formulation were accomplished. The mean and standard deviation (SD) of dissolved percentages were calculated.

2.4. In vivo absorption studies

The animal experimental protocol was approved by the Institutional Animal Care and Use Committee of Kaohsiung Medical University. The committee confirmed that the animal experiment had followed the guidelines as set forth by the Guide for Laboratory Fact lines and Care. All of the dogs were fasted 12 hours prior to the experiment, but water drinking was not limited. Their legs were pre-shaved, and a forefoot vein was cannulated using an 18-gauge cannula. Blood samples (3 mL) were collected in a heparin tube at the

Table 1 – Propranolol extended-release tablets used in the development and validations of *in vitro*–*in vivo* correlation IVIVC.

	Formulations		
	ER-F	ER-S	ER-V
BioStudy	Internal	Internal	External
Ingredients (%) of tablets			
HPMC	28.0	60.0	38.4
Avicel	26.2	15.0	5.8
Lactose	20.8	0.0	27.3

Each tablet contains propranolol 100 mg.
HPMC = hydroxypropyl methylcellulose.

following time intervals: 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, and 24 hours, after administering the experimental ER tablets (100 mg) and commercial immediate-release (IR) tablets (40 mg, Astrazeneca, London, UK) orally. Each treatment was separated by a washout period of at least 1 week. The blood samples were centrifuged at 3000 rpm for 10 minutes, and the plasma was taken and kept frozen for further analysis. The plasma drug concentration was determined according to a previous method [12]. A Merck (Darmstadt, Germany) Lichrocart C18 column (250 × 4 mm I.D., particle size 5 μm) was used. The mobile phase was a mixture of acetonitrile and a pH 3.0 acetic acid aqueous solution at ratio of 25:75, at a flow rate of 1 mL/minute. The detection wavelength was set to 230 nm and the excitation wavelength to 340 nm. The concentration, ranging from 5 ng/mL to 1000 ng/mL, showed a linearity ($r = 0.996$). The coefficients of variation (CV%, $n = 6$) ranged from 0.64% to 17.82% for the interday and intraday analyses. The lower limit of quantitation was 5 ng/mL.

2.5. In vitro data analysis

Dissolution analyses for each tablet were conducted by plotting the cumulative percentage of the propranolol dissolved at different time points. The *in vitro* drug release profiles of the ER formulations were compared using the similarity factor f_2 [13]. An f_2 value >50 (50–100) represents similarity or equivalence of the two curves.

2.6. In vivo data analysis

The pharmacokinetic parameters of the reference product for developing the IVIVC, namely, the volume of distribution (V_d) and elimination rate constant (K_e), were obtained from the IR product by fitting a one-compartment model using WINNONLIN software (Pharsight Corporation, Mountain view, CA, USA). Then, the numerical deconvolution analysis provided by WINNONLIN was used to acquire % *in vivo* cumulative absorption input profiles of propranolol from the ER tablets. A mathematical model (IVIVC Mode), which related the *in vitro* cumulative dissolution data release to the fraction of drug absorbed, was developed by linear regression. The relationship of *in vitro* cumulative dissolution data and *in vivo* cumulative absorption data is shown in Eq. 1.

$$\% \text{ in vivo cumulative input (t)} = \alpha + \beta \times \% \text{ in vitro cumulative dissolved (t)} \quad (1)$$

where the data for “% *in vivo* cumulative input (t)” was obtained from the numerical deconvolution approach and “% *in vitro* cumulative dissolved (t)” from the experimental data. The intercept, slope, and determination coefficient of the regression line are denoted by α , β , and r^2 , respectively.

2.7. Evaluation of the IVIVC model

The validation of the IVIVC model can be accomplished by using the internal and/or external predictabilities. The internal predictability involves the use of the initial data used to define the IVIVC model. Hence, the predicted plasma concentration

profiles of formulation ER-S and ER-F were calculated using their *in vitro* dissolution data. For the external predictability, an additional formulation ER-V with an *in vitro* moderate release rate between formulation ER-S and formulation ER-F was prepared. The *in vitro* dissolution test and bioavailability study of formulation ER-V were conducted. The predicted plasma concentration profile of formulation ER-V was calculated by using its dissolution data based on the IVIVC model.

The “predicted % *in vivo* cumulative input (t)” data were calculated using the intercept, slope, and “% *in vitro* cumulative dissolved (t)” data. In other words, the “% *in vivo* cumulative input (t) data” versus time profile was estimated from the “% *in vitro* cumulative dissolved (t) data” based on an established IVIVC model. Subsequently, the predicted *in vivo* input rates for the ER formulation were obtained from the “% *in vivo* cumulative input (t) data” as shown in Eq. 2.

$$R_{\text{input}} = \left\{ \frac{\% \text{ in vivo input (t}_2) - \% \text{ in vivo input (t}_1)}{t_2 - t_1} \right\} \times \text{dose} \quad (2)$$

where “ R_{input} ” is the “predicted *in vivo* input rates” and “% *in vivo* input (t)” is the input value at the time point t .

Then, according to Eq. 3, the unit disposition function [14], “the predicted *in vivo* input rates” was converted to the predicted plasma concentration-time profiles using Microsoft Excel 2010 (Microsoft corporation, Washington, USA).

$$C_p(t) = \sum_{j=1}^m \left[\frac{R_j}{\lambda} \left\{ e^{-\lambda(t-\tau_2)} - e^{-\lambda(t-\tau_1)} \right\} \right] \quad (3)$$

where $C_p(t)$ is the predicted plasma concentration at time point t ; R_j is the j -th input rate; τ_1 is the start time of the j -th input rate; and τ_2 is the stop time of the j -th input rate. In addition, the unit disposition function parameters were obtained by fitting a one-compartment model to the IR formulation plasma concentration-time profile. C and λ were calculated as the reciprocal of V_d and K_e , respectively, for a one-compartment model (mono exponential).

The absolute percent prediction error (%PE) values for C_{max} and $AUC_{0-\infty}$ are calculated as follows:

$$\%PE = \frac{P_{\text{obs}} - P_{\text{pred}}}{P_{\text{obs}}} \quad (4)$$

where P_{obs} and P_{pred} are the observed and IVIVC model-predicted values for C_{max} and $AUC_{0-\infty}$, respectively. The IVIVC is considered valid if the averaged absolute %PE is not more than 10% and if the %PE for each formulation is not more than 15%.

3. Results and discussion

3.1. In vitro studies

The mean dissolution profiles of propranolol ER tablets ER-F and ER-S are shown in Fig. 1A. The calculated similarity

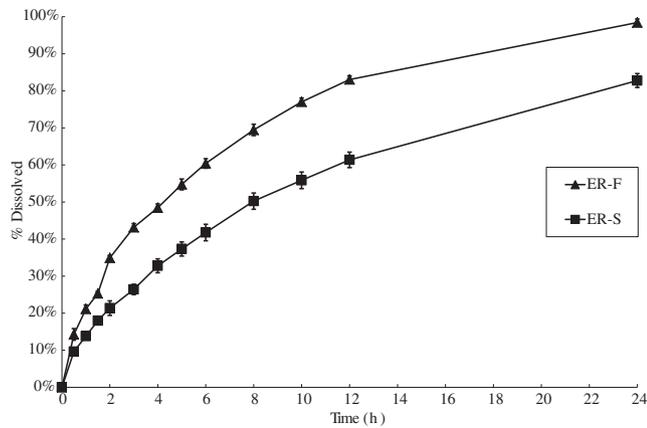


Fig. 1 – In vitro dissolution profiles of propranolol hydrochloride from different release rates formulations extended-release tablet ER-F (▲) and ER-S (■).

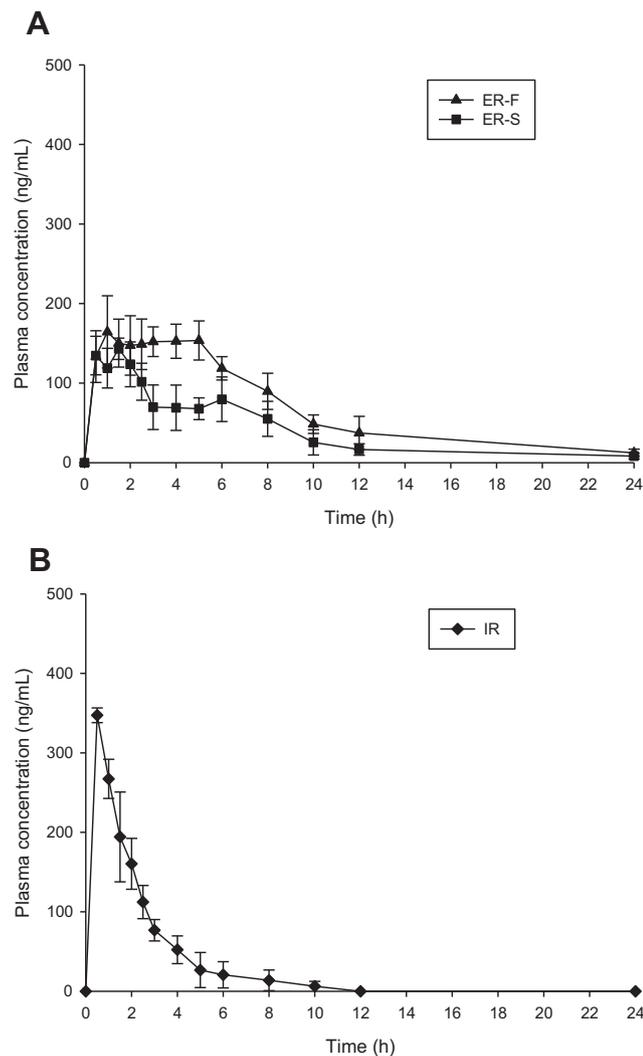


Fig. 2 – Mean propranolol plasma concentrations versus time profiles of formulation (A) extended-release tablets of ER-F (▲) and ER-S (■); (B) immediate-release commercial products (IR) (◆).

Table 2 – Mean pharmacokinetic parameters of propranolol extended-release (ER) tablets and immediate-release (IR) commercial product.

Formulations	C_{\max} (ng/mL)	T_{\max} (h)	$AUC_{0-\infty}$ (ng \times h/mL)
ER-F	140.82 \pm 23.85	1.88 \pm 1.44	1059.12 \pm 128.90
ER-S	78.83 \pm 31.42	1.50 \pm 0.41	955.35 \pm 159.89
IR	347.33 \pm 9.28	0.50 \pm 0.00	758.06 \pm 199.19

$AUC_{0-\infty}$ = area under the curve from time zero to infinity;
 C_{\max} = maximum observed plasma propranolol concentration;
 T_{\max} = time to C_{\max} .

factor (f_2) value was 38.1 between these two ER tablets. The value of f_2 was <50 , indicating dissimilarity between the curves. The wide variation indicated that the combinations component of formulation (Table 1) resulted in different drug release rates. Among the components, HPMC formed a

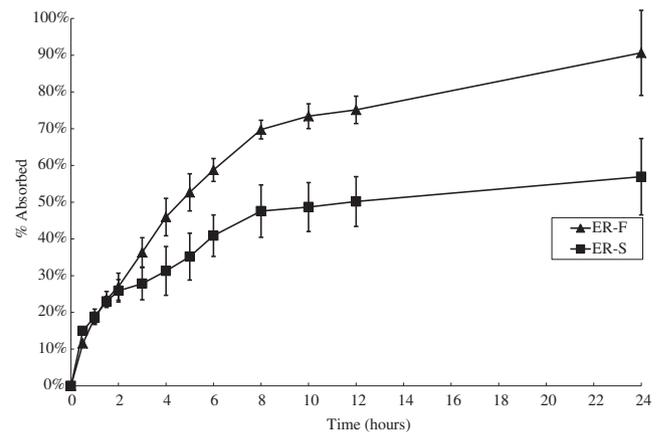


Fig. 3 – Mean cumulative percentage absorption rate of propranolol of extended-release formulations ER-F (▲) and ER-S (■) calculated by using the numerical deconvolution approach.

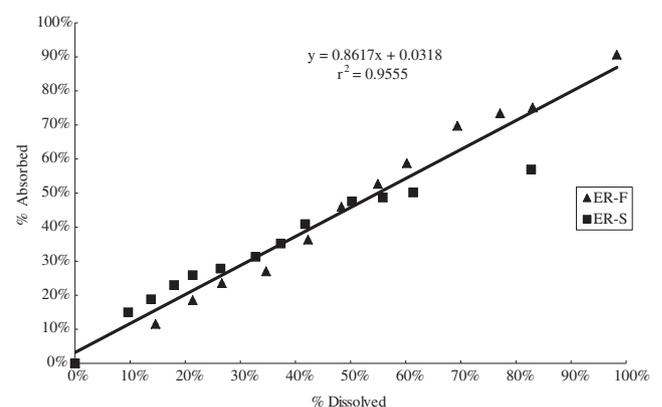


Fig. 4 – In vitro–in vivo correlation (IVIVC) model linear regression plots ($r^2 = 0.9555$) of “% dissolved” versus “% absorbed” for extended-release formulations ER-F (▲) and ER-S (■).

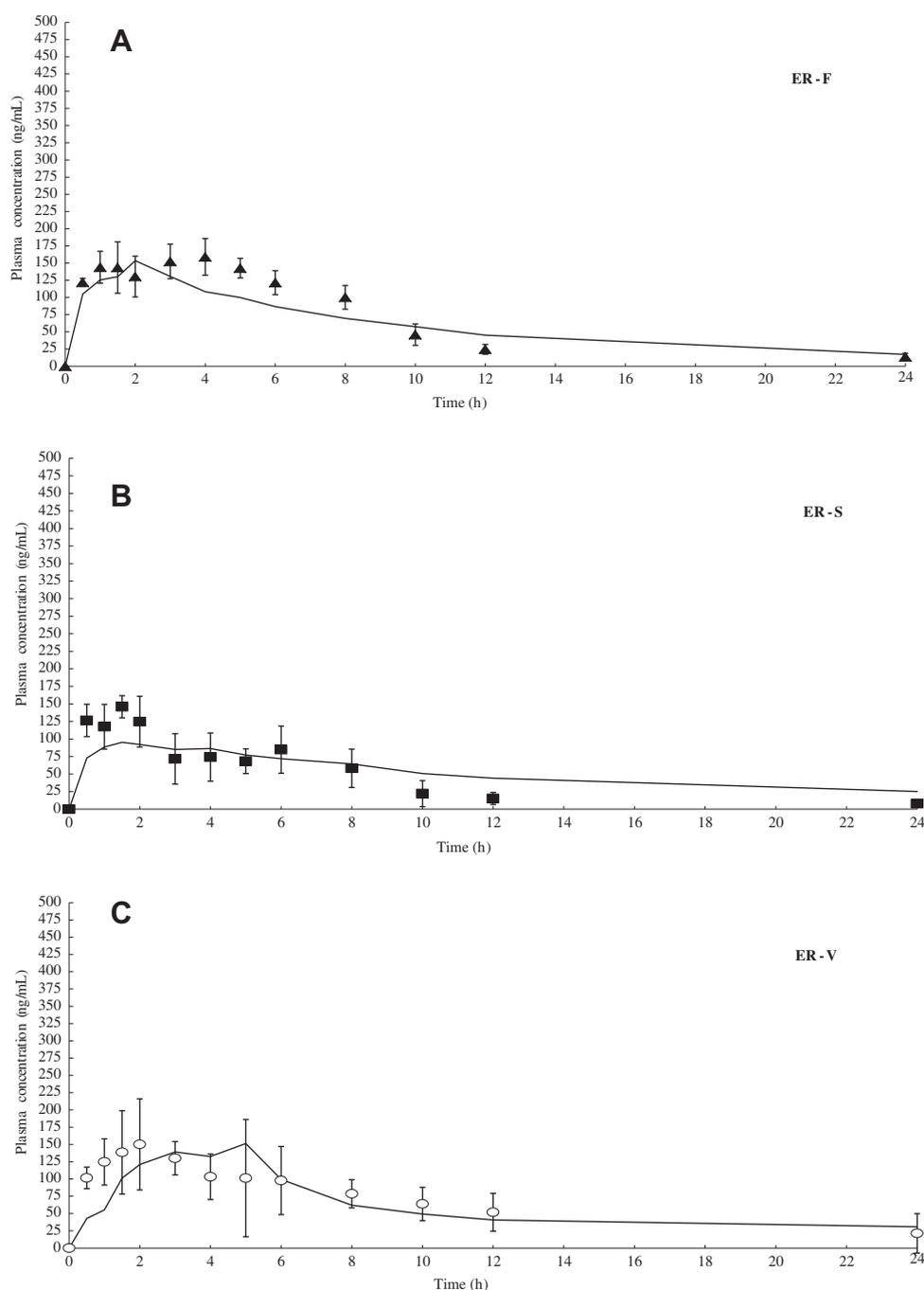


Fig. 5 – Mean observed (symbols) and predicted (solid line) propranolol plasma concentrations versus time profiles of extended-release formulations ER-F (\blacktriangle) and ER-S (\blacksquare) and ER-V (\circ). Error bars displayed standard deviations of observed data.

hydrogel matrix, and Avicel and lactose serve as fillers. From the *in vitro* dissolution profile shown in Fig. 1, the release rate of ER-S was much slower than those of other formulations. In the ER-S formulation, there is no lactose added. Based on a previous study by Huang et al [11], lactose incorporated into the gel-forming matrix could improve the phenomenon in *in vitro* and/or *in vivo* studies, because these excipients could stimulate the water penetration into the inner parts of the matrix, thus resulting in the drug release from the matrix. The

lactose inside the matrix would dissolve and became porous and the dissolution rate was increased.

For the external validation study, an additional propranolol ER formulation ER-V, with a different release rate from ER-F and ER-S, was prepared and subjected to a dissolution test. The f_2 values of the formulation ER-F/ER-V and the formulation ER-S/ER-V were 76.6 and 40.3, respectively, indicating that the drug release rate from formulation ER-V was different to that from the ER-F and ER-S formulations.

3.2. In vivo studies

The plasma concentration profiles of ER dosage formulations as well as that of the IR dosage formulation are shown in Fig. 2. The mean pharmacokinetic parameters, C_{\max} , T_{\max} , and $AUC_{0-\infty}$ are summarized in Table 2. The $AUC_{0-\infty}$ and C_{\max} values were $1509.12 \pm 128.90 \text{ ng} \times \text{h/mL}$ and $140.82 \pm 23.85 \text{ ng/mL}$ for formulation ER-F and $955.35 \pm 159.89 \text{ ng} \times \text{h/mL}$ and $78.83 \pm 31.42 \text{ ng/mL}$ for formulation ER-S, respectively. It was found that T_{\max} values of ER tablets (ER-S and ER-F) were prolonged when compared with the IR commercial products. The T_{\max} values of ER-F and ER-S were not significantly different. The rank order of C_{\max} and AUC of ER tablets was reflected in the drug release rate observed in dissolution test.

3.3. The development of the IVIVC model

The *in vivo* cumulative absorption fraction profiles of propranolol ER tablets of ER-S and ER-F calculated by the numerical deconvolution analysis are shown in Fig. 3. It was found that the same rank order was observed between the *in vitro* release rate of dissolution (Fig. 1) and the *in vivo* release rate of absorption (Fig. 3), i.e., formulation ER-F > formulation ER-S. The IVIVC model was developed by using the linear regression based on the “% *in vitro* cumulative dissolved (t)” and “% *in vivo* cumulative input (t)” of formulation ER-F and ER-S (Fig. 4). The determination coefficient r^2 was 0.9555, indicating a good linear correlation for the IVIVC model ($p < 0.05$). The intercept and slope were $\alpha = 0.0318$ and $\beta = 0.8517$, respectively. The slope of 0.8517 was lower than 1, indicating that the *in vivo* absorption rate was underestimated. The phenomenon might be attributed to the hepatic first pass effect of propranolol [10].

3.4. Validation of the IVIVC model

The internal validation was accomplished by convolving the dissolution data corresponding to the formulations ER-S and ER-F. Fig. 5 shows the observed and predicted plasma concentrations for formulation ER-F (Fig. 5A) and formulation ER-S (Fig. 5B), respectively. Table 3 represents the %PE values for the C_{\max} and $AUC_{0-\infty}$ of these two ER formulations. The %PE values of the C_{\max} and $AUC_{0-\infty}$ for formulation ER-S were 36.80% and -23.00%, respectively. The %PE values of the C_{\max} and $AUC_{0-\infty}$ for formulation ER-F were 3.45% and -4.92%, respectively. The average absolute %PE values of C_{\max} and $AUC_{0-\infty}$ were 13.9% and 20.1%, respectively. The FDA guidelines [1] state that the predictability can be accepted when the average absolute %PE

values are of 10% or less and the %PE value for each formulation should not exceed 15%. In the present study, the average absolute %PE values of $AUC_{0-\infty}$ and C_{\max} for the formulation ER-F and ER-S were >10%. The failure of the IVIVC model to predict the extent of propranolol hydrochloride absorption may be explained by the first-pass metabolism. The *in vitro* dissolution data presented the total dose; however, the *in vivo* absorption data presented the fraction of the active drug reaching the systemic circulation. Because the predicted $AUC_{0-\infty}$ involved converting the *in vitro* dissolution profile into the *in vivo* dissolution profile, the predicted $AUC_{0-\infty}$ values of the formulation ER-F and ER-S might be higher than the observed $AUC_{0-\infty}$ values. Thus, if we utilized the *in vitro* dissolution profiles to predict the *in vivo* absorption, we should consider the factor of first-pass metabolism. The previous studies have reported that first-pass metabolism played an important role in the assessment of bioequivalence [15–17]. Sirisuth and Eddington [10] proved that the influence of the first-pass metabolism of an IVIVC model on metoprolol ER tablets in which metoprolol displayed a high extraction ratio, was the same as for propranolol. Furthermore, Keller et al [18] reported that the bioavailability of long-acting propranolol decreased with the drug absorption rate (K_a). Hence, the high %PE value of the $AUC_{0-\infty}$ of formulation ER-S may contribute to the decrease of the drug absorption rate.

The FDA guidelines [1] declare that if the criteria for internal validation are not met, the external predictability should be evaluated as the final determination of the IVIVC model. Then, the *in vitro* dissolved rate and *in vivo* absorbed rate of an additional ER formulation, ER-V, was used to examine the ability of the IVIVC model and to predict the *in vivo* performance. Fig. 5C shows the observed and predicted plasma concentration profiles. The %PE values of the C_{\max} and $AUC_{0-\infty}$ for formulation ER-V were 0.86% and -5.95% (Table 3), respectively. An absolute %PE value of 10% or less for C_{\max} and $AUC_{0-\infty}$ validated the external predictability of an IVIVC model, which indicated that the results met the criteria of IVIVC guidelines for good external predictability [1]. This demonstrates that the IVIVC model could be used as a surrogate for bioequivalence.

4. Conclusions

In this preliminary study, a level A IVIVC model for propranolol ER dosage formulations was developed and estimated for both internal and external predictability. Although the model was not acceptable for validating internal predictability, validation of external predictability was achieved. Thus, this IVIVC model might be used to predict the variation in site change, process changes, scale-up, and to predict the absorption performance of propranolol hydrochloride products with different release rates.

REFERENCES

- [1] Center for Drug Evaluation and Research (CDER), USFDA. Guidance for industry—extended release oral dosage forms: development, evaluation, and application of *in vitro/in vivo* correlations; 1997.

Table 3 – Percent prediction errors (%PE) associated with C_{\max} and $AUC_{0-\infty}$.

Formulations	C_{\max}	$AUC_{0-\infty}$
Internal predictability		
ER-F	3.45%	-4.92%
ER-S	36.80%	-23.00%
Average absolute %PE	20.13%	13.96%
External predictability		
ER-V	0.86%	-5.95%

- [2] Center for Drug Evaluation and Research (CDER), USFDA. Guidance for industry—SUPAC-MR: modified release solid oral dosage forms: scale-up and postapproval changes: chemistry, manufacturing and controls, *in vitro* dissolution testing and *in vivo* bioequivalence documentation; 1997.
- [3] Eddington ND, Ashraf M, Augsburger LL, et al. Identification of formulation and manufacturing variables that influence *in vitro* dissolution and *in vivo* bioavailability of propranolol hydrochloride tablets. *Pharm Dev Technol* 1998;3:535–47.
- [4] Lu Y, Kim S, Park K. *In vitro-in vivo* correlation: perspectives on model development. *Int J Pharm* 2011;418:142–8.
- [5] Hayes S, Dunne A, Smart T, et al. Interpretation and optimization of the dissolution specifications for a modified release product with an *in vivo-in vitro* correlation (IVIVC). *J Pharm Sci* 2004;93:571–81.
- [6] Emami J. *In vitro-in vivo* correlation: from theory to applications. *J Pharm Pharm Sci* 2006;9:169–89.
- [7] Mahayni H, Rekhi GS, Uppoor RS, et al. Evaluation of “external” predictability of an *in vitro-in vivo* correlation for an extended-release formulation containing metoprolol tartrate. *J Pharm Sci* 2000;89:1354–61.
- [8] Mandal U, Ray KK, Gowda V, et al. *In-vitro* and *in-vivo* correlation for two gliclazide extended-release tablets. *J Pharm Pharmacol* 2007;59:971–6.
- [9] Mendyk A, Tuszyński PK, Polak S, et al. Generalized *in vitro-in vivo* relationship (IVIVR) model based on artificial neural networks. *Drug Des Devel Ther* 2013;7:223–32.
- [10] Sirisuth N, Eddington ND. The influence of first pass metabolism on the development and validation of an IVIVC for metoprolol extended release tablets. *Eur J Pharm Biopharm* 2002;53:301–9.
- [11] Huang YB, Tsai YH, Yang WC, et al. Once-daily propranolol extended-release tablet dosage form: formulation design and *in vitro/in vivo* investigation. *Eur J Pharm Biopharm* 2004;58:607–14.
- [12] Semple HA, Xia F. Simplified high-performance liquid chromatographic method for propranolol and five metabolites in liver perfusate, rat serum and dog plasma. *J Chromatogr B Biomed Appl* 1994;655:293–9.
- [13] Center for Drug Evaluation and Research (CDER), USFDA. Guidance for industry—dissolution testing of immediate release and solid oral dosage forms; 1997.
- [14] Dutta S, Qiu Y, Samara E, et al. Once-a-day extended-release dosage form of divalproex sodium III: development and validation of a Level A *in vitro-in vivo* correlation (IVIVC). *J Pharm Sci* 2005;94:1949–56.
- [15] Chen ML, Jackson AJ. The role of metabolites in bioequivalency assessment. I. Linear pharmacokinetics without first-pass effect. *Pharm Res* 1991;8:25–32.
- [16] Chen ML, Jackson AJ. The role of metabolites in bioequivalency assessment. II. Drugs with linear pharmacokinetics and first-pass effect. *Pharm Res* 1995;12:700–8.
- [17] Jackson AJ. The role of metabolites in bioequivalency assessment. III. Highly variable drugs with linear kinetics and first-pass effect. *Pharm Res* 2000;17:1432–6.
- [18] Keller F, Kunzendorf U, Walz G, et al. Saturable first-pass kinetics of propranolol. *J Clin Pharmacol* 1989;29:240–5.