Influence of Stereoselective Pharmacokinetics in the Development and Predictability of an IVIVC for the Enantiomers of Metoprolol Tartrate

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Purpose. To investigate the ability of an IVIVC developed with a racemate drug as well as each enantiomer in predicting the *in vivo* enantiomer drug performance.

Methods. Dissolution of metoprolol extended release tablets with different release characteristics (e.g., fast (**F**), moderate (**M**), and slow (**S**)) was performed using USP Apparatus I, pH 1.2, 50 rpm. Metoprolol racemate tablets (S, M, and F, 100 mg) and 50 mg oral solution were administered to healthy volunteers, blood samples were collected over 24 (solution) and 48 (tablet) hours and assayed. IVIVC models developed were: (1) Racemate-fraction of drug dissolved (FRD) vs Racemate-fraction of drug absorbed (FRA), (2) R-FRD vs R-FRA, and (3) S-FRD vs S-FRA for combinations of formulations (S/M/F, S/M, S/F, and M/F). Enantiomer Cmax and AUC prediction errors (PEs) were estimated for model evaluation after convolution of *in vivo* release rates.

Results. The R-IVIVC and S-IVIVC accurately predicted the R- and S-metoprolol pharmacokinetic profiles, respectively. The averaged prediciton errors (PE) for the enantiomer Cmax and AUC were less than 10% for S/M/F, M/F, and S/F IVIVC models. Racemate-IVIVC (M/F) was able to predict S-enantiomer with an average %PE of 2.52 for S-Cmax and 4.3 for S-AUC. However, the racemate-IVIVC was unable to predict the R-enantiomer pharmacokinetic profile.

Conclusions. Metoprolol racemate data cannot be used to accurately predict R-enantiomer drug concentrations. However, the racemate data was predictive of the active stereoisomer.

KEY WORDS: IVIVC; racemate; enantiomers; metoprolol; pharmacokinetics.

INTRODUCTION

A validated *in vitro in vivo* correlation (IVIVC) may facilitate product development, since it has the potential of predicting the pharmacokinetic profile of a formulation without the necessity of biostudies. The main objective of developing and evaluating an IVIVC is to enable the dissolution test to serve as a surrogate for *in vivo* behavior. Recommendations for formulation and manufacturing changes as well as the process of IVIVC development and validation are outlined in the Scale-up and Post Approval Changes—Modified Release (SUPAC-MR) and the IVIVC guidances, respectively (1,2). However, these documents do not consider the implication of stereoisomerisms in the development of an IVIVC.

Stereoselectivity may influence both drug absorption and disposition kinetics (3–5). In addition, for some racemic drugs, the inherent pharmacological activity is associated with only one enantiomer (4–8). Nonetheless, metabolic differences between enantiomers may be more pronounced for an isomer that exhibits a high extraction rate. Thus, the bioavailability of enantiomers may be significantly different, due to preferential first pass metabolism (4,6,7). This difference may not be reflected in the bioavailability profile of the racemic drug and the racemate profile may not provide an accurate surrogate of the therapeutic effect (3). This suggests that IVIVC development for extended release formulations using racemic drug data alone may not accurately predict the *in vivo* availability of the "active" enantiomer, and hence the therapeutic effect of the active form. In such cases, consideration of stereoisomerisms in the IVIVC development process may provide a more meaningful relationship and better relate to *in vivo* response.

The development and validation of an IVIVC for metoprolol has been reported (9). However, the influence of stereoisomerisms has not been addressed. Metoprolol is marketed as the racemate drug, and the S-isomer is pharmacoligically active. The elimination of S-metoprolol is slower than R-metoprolol in extensive metabolizers (4). Moreover, the magnitude of the difference in plasma concentration of R-and S-metoprolol in extensive metabolizers is greater after slow drug input. The prediction of the bioavailability parameters, Cmax and AUC, based on an IVIVC developed with racemate data may not accurately describe the bioavailability of S-metoprolol.

The underlying assumption in the use of IVIVCs is that the correlation is predictive of the *in vivo* behavior of the active species and plasma drug concentrations are related in some manner to drug effect. This work explored the ability of an IVIVC developed with the racemate drug as well as each enantiomer in predicting the *in vivo* enantiomer drug performance. In the present study, the racemate and R and S-enantiomers of metoprolol were employed to establish IVIVCs. The ability of the IVIVCs to predict the *in vivo* behavior of both the R- and S-enantiomer was evaluated.

MATERIALS AND METHODS

Materials

Metoprolol tartrate ER tablets were obtained from the Industrial Pharmacy Laboratory, University of Maryland. Metprolol and atenolol standard compounds were purchased from Sigma Chemical Co. (St. Louise, MO). Optically pure R-and S-metoprolol were provided by Astra (Sweden). Potassium Chloride and glacial acetic acid were purchased from JT. Baker Chemical Co. (Philipsburge, New Jersey). All chemical and solvents were HPLC grade.

Formulations

Extended release formulations of metoprolol were manufactured at the Industrial Pharmacy Laboratory at the University of Maryland using hydroxypropyl methylcellulose (HPMC) as the release rate controlling excipient. The formulations were designed to release metoprolol (100 mg) at three different rates referred to as: slow (S), moderate (M) and fast (F) [~24, 15 and

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10%/hr, respectively]. Metoprolol extended release formulation development and manufacturing have been previously reported elsewhere (9,10).

Dissolution

The release characteristics of the formulations were examined using the following dissolution testing methodologies: (1) Apparatus I, pH 6.8, 150 rpm, (2) Apparatus II, pH 6.8, 50 rpm, and (3) Apparatus II, pH 1.2, 50 rpm (11). Two dissolution media, pH 1.2 and pH 6.8, without the enzyme were prepared. Dissolution samples were collected at the following times: 0, 0.5, 1, 1.5, 2, 3, 4, 5, 6, 8, 10 and 12 hours. Dissolution tests were performed on six tablets and the amount of metoporlol racemate released was analyzed spectrophotometrically at a wavelength of 275 mm. An enantiomeric assay was used to quantitate the R-and S-enantiomer released.

Bioavailability Study

The bioavailability study has been previously reported (9). Briefly, this was an open, fasting, single dose, four treatment crossover study using normal healthy volunteers. The debrisoquin-type metabolizing capabilities of each subject was determined by dextromethorphan screening and only extensive metabolizers were enrolled (12). Seven normal healthy, male and female, non-smoking volunteers were enrolled in the study and received three formulations (S, M, and F) of racemic metoprolol (100 mg) in a randomized fashion. In addition to the extended release formulations, an oral solution (50 mg) of racemic metoprolol tartrate was also administered. Blood samples (6 ml) were collected over a 24 hour period after the administration of each treatment. Samples were centrifuged for 10 minutes at 25°C and subsequently stored at −80°C until assayed.

Racemate and Enantiomer Assay Method

Plasma and dissolution samples were analyzed for the racemate, R- and S-metoprolol using a valid High Performance Liquid Chromatography (HPLC) with fluorescence detection (12,13). Chromatography involved direct separation of enantiomers using a Chirobiotic T™ bonded phase column (250 × 4.6 mm) and a mobile phase consisting of ACN/MeOH/$MeCl_2$/glacial acetic acid/triethylamine [56/30/14/2/2 (v/v/v/v)]. Solid phase extraction using silica bonded with ethyl group (C_2) was used to extract the compounds of interest from plasma and atenolol was used as the internal standard. The column effluent was monitored using fluorescence detection with excitation and emission wavelengths of 225 nm and 310 nm, respectively. The mean intra-run and inter-run accuracies were in the range of 96.2 to 114% and 97.1 to 106% for R-metoprolol, and 94.0 to 111% and 99.3 to 106% for S-metoprolol, respectively. The lowest level of quantitation for the enantiomers of metoprolol was 0.5 ng/ml.

In Vitro Dissolution Data Analysis

The dissolution profiles for each formulation (S, M, and F) were determined by plotting the cumulative fraction of the metoprolol racemate dissolved (racemate-FRD) at various time points. The cumulative fraction of R-metoprolol (R-FRD) and S-metoprolol (S-FRD) was also determined for each formulation. The dissolution data were mathematically modeled by fitting the mean profiles of racemate, R-, and S- enantiomers to the following Hill equation:

$$\%\text{Dissolved} = \frac{D\ \max^{*}\ T^{\gamma}}{D_{50}^{\gamma} + T^{\gamma}} \quad (1)$$

where % Dissolved is the % drug dissolved at time T, D_{max} = the maximum (cumulative) % drug dissolved, D_{50} = the time required for 50 % of the drug to dissolve, T = time and γ = the sigmoidicity factor.

The *in vitro* drug release profiles were compared using the similarity factor, f_2, presented in the following equation (14):

$$f_2 = 50 \log \{[1 + 1/n \sum_{t=1}^{n} (R_t - T_t)^2]^{-0.5} \times 100\} \quad (2)$$

where R_t and T_t are the percent dissolved at each time point for the reference and test products and n is the number of pooled points.

Pharmacokinetic Analysis

Metoprolol mean plasma profiles of the enantiomers or racemate for the S, M, and F formulations, and the oral solution were modeled using WinNonlin software (Scientific Consulting Inc., NC). Various models and weighting factors were used to minimize the sum of squares residual value between the observed and model predicted plasma drug concentrations. The R, S, and racemate oral solution profiles were fit to a first-order absorption one compartment model without lag-time, while the tablet formulations were fit to first-order absorption one compartment models that included lag-time. These models provided the lowest AIC value. The pharmacokinetic parameters estimated were Cmax, Tmax, AUC, Ka, λ_z, and V/F

Deconvolution of Plasma Concentration Profiles

The metoprolol fraction absorbed vs. time profiles were estimated using numerical deconvolution (PCDCON software). The impulse response was the plasma drug concentrations associated with the oral solution and the input response was the plasma drug concentration profiles of the respective formulations. The fraction of drug absorbed was determined for racemic metoprolol (racemate-FRA), R-metoprolol (R-FRA) and S-metoprolol (S-FRA). The racemic data were dose normalized for equivalency to the enantiomer dose (50% of the racemate) and plasma drug concentrations.

IVIVC Model Development

Linear correlations between pooled fraction of drug dissolved (racemate-FRD, R-FRD or S-FRD) and pooled fraction of drug absorbed (racemate-FRA, R-FRA or S-FRA) were developed for the following combinations of formulations: slow/moderate/fast (S/M/F), slow/moderate (S/M), slow/fast (S/F) and moderate/fast (M/F). The correlations performed included: (1) racemate-FRD vs racemate-FRA, (2) S-FRD vs S-FRA and (3) R-FRD vs R-FRA. In addition, the correlations were developed for all dissolution testing conditions. A linear regression using ordinary least squares method was applied to estimate the regression parameters. The F-statistic was used

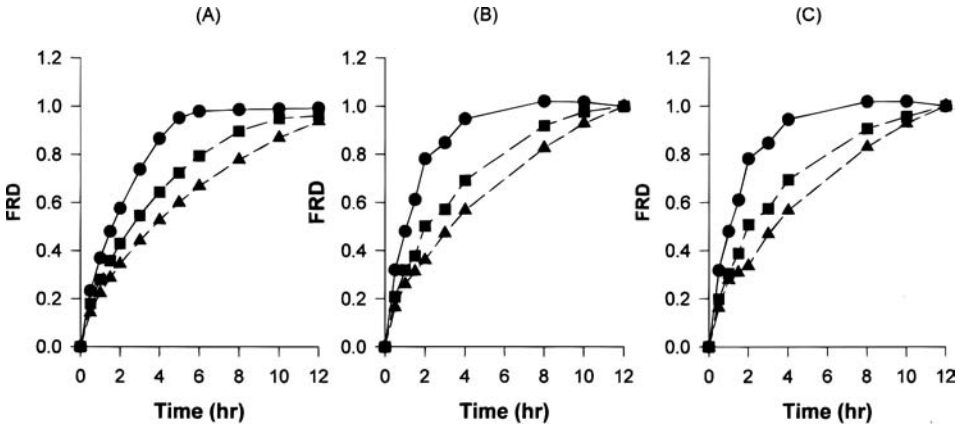

Fig. 1. Mean dissolution versus time profiles for (A) racemate, (B) R-metoprolol, and (C) S-metoprolol after the administration of the slow (▲), moderate (■), and fast (●) using Apparatus II, pH 1.2, 50 rpm.

to determine if a slope was significantly different form one ($p < 0.05$).

Predictability of the IVIVC

The internal validity of the IVIVC models was evaluated to determine how well the correlations predicted the *in vivo* behavior of the enantiomers after administration of the various formulations. The IVIVC model from all formulations (F/M/S) was used to predict the *in vivo* performance of the F, M, and S formulations. Cross validation was also used in this study. The IVIVC obtained from any two formulations was employed to predict the *in vivo* plasma profiles of the remaining formulation, such as using F/M-IVIVC in predicting the S formulation or M/S-IVIVC in predicting the F formulation.

Since the IVIVC is used to serve as a surrogate of the *in vivo* behavior, the predictability of Cmax and AUC for the enantiomers was determined. The *in vivo* enantiomer plasma profile was estimated based on the convolution integral. Briefly, the *in vitro* dissolution rates were determined by taking the first derivative of the cumulative amount of drug dissolved. It then was converted to *in vivo* dissolution rate by using the IVIVC regression parameters. The predicted plasma concentration corresponding to its *in vivo* dissolution rate was accomplished by convolution of the *in vivo* dissolution rate and the pharmacokinetic model for the oral solution. Cmax and AUC prediction errors (PE) were obtained. The IVIVC was considered valid if the averaged absolute % prediction error was ≤ 10 for Cmax and AUC and the % prediction error for each formulation did not exceed 15%.

RESULTS

In Vitro Dissolution

Suitable dissolution testing conditions for developing the R-IVIVC were the basket method at pH 6.8 and 150 rpm and paddle method at pH 1.2 and 50 rpm. The suitable condition for S-IVIVC development was the paddle method at pH 1.2 and 50 rpm. Dissolution testing using apparatus II, pH 1.2 (50 rpm) was more representative of the enantiomeric *in vivo* absorption profiles and for this reason linear regression relationships were developed using this system. The %CV of dissolution profiles for each formulation (fast, moderate, slow) was less than 10 for both conditions suggesting a consistent drug release. The similarity factor indicated dissimilarity between pairs of the study formulations ($f_2 < 50$). Figures 1A–1C illustrate mean dissolution profiles of racemate (Fig. 1A), R- metoprolol

Table I. Mean (SD) Pharmacokinetic Parameters for R- metoprolol (R) and S-metoprolol (S) After the Administration of Extended Release Metoprolol Racemate Tablets (n = 7)

	Pharmacokinetic parameter					
Formulation	V/F (l)	Ka (hr^{-1})	Kel (hr^{-1})	T_{max} (hr)	C_{max} (ng/ml)	AUC (ng.hr/ml)
Fast						
R	638 (131)	0.85 (0.24)	0.24 (0.03)	2.85 (0.16)	49.3 (13.0)	344 (77)
S	440 (103)	0.72 (0.31)	0.24 (0.05)	3.05 (0.23)	66.1 (17.1)	507 (112)
Moderate						
R	753 (245)	0.50 (0.12)	0.21 (0.03)	3.99 (0.98)	37.2 (12.4)	338 (101)
S	538 (131)	0.41 (0.11)	0.21 (0.04)	3.84 (0.45)	49.7 (12.2)	472 (116)
Slow						
R	997 (351)	0.27 (0.10)	0.19 (0.05)	4.43 (1.13)	28.3 (7.2)	294 (75)
S	622 (132)	0.30 (0.07)	0.20 (0.05)	4.52 (1.19)	40.3 (9.6)	441 (121)

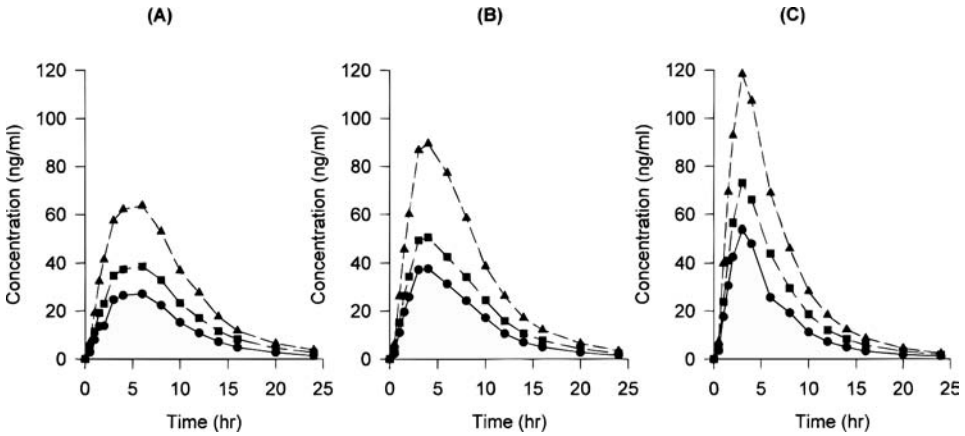

Fig. 2. Mean plasma concentrations versus time profile for the racemate (▲), R-metoprolol (●), and S-metoprolol (■) after the administration of the (A) slow, (B) moderate, and (C) fast formulations.

(Fig. 1B), and S- metoprolol (Fig. 1C) for the **F**, **M**, and **S** formulations using the paddle condition. R- and S-dissolution profiles were similar. The racemate profiles, however, were slightly different from the enantiomers.

In Vivo Studies

Mean pharmacokinetic parameters for R- and S-enantiomers for fast, moderate and slow release formulations are summarized on Table I. It was found that the S-AUC and S-Cmax were higher than those of the R- enantiomer for all study formulations. Figure 2 presents the mean plasma drug concentration vs. time profile for the racemate, R- metoprolol and S-metoprolol after the administration of the slow, moderate and fast formulations. Figures 3A–3C present graphical comparisons of the fraction of drug absorbed for the racemate, S-metoprolol and R-metoprolol after the administration of the slow (Fig. 3A), moderate (Fig. 3B) and fast (Fig. 3C) formulations.

IVIVC Development

The regression lines obtained between FRA and FRD for all IVIVC models were found to be significant at a high probability and slope was found significant different from 1 ($p < 0.05$). Figures 4A–4C present the **S**/**M**/**F** IVIVC model linear regression plots of FRD vs FRA for racemate and each enantiomer.

IVIVC Predictability

Table II presents the enantiomer Cmax and AUC averaged prediction errors for the correlation models obtained from the racemate-IVIVC, R-IVIVC and S-IVIVC. The averaged R-Cmax prediction errors for the racemate-IVIVC ranged from 38.2–54.0 % and the corresponding R-AUC ranged from 42.0–60.1%. Figures 5A–5C present the observed and racemate-IVIVC model predicted plasma R-metoprolol profiles for the correlation developed with the S/M/F formulation. Neither the rate nor extent of absorption of the R-enantiomer was predicted by the IVIVC developed with the racemate.

The IVIVCs developed solely with the R-FRD and R-FRA accurately characterized in the *in vivo* behavior of R-metoprolol. The range of averaged prediction errors for R-Cmax and R-AUC with this model was 4.70–11.50% and 6.6–17.10%, respectively (Table II). Figures 6A–6C present the observed and R-IVIVC model predicted R-metoprolol profiles for the correlation developed with the S/M/F formulation. The IVIVCs developed with the S/M/F, M/F, and S/F formulations accurately described the R-metoprolol plasma levels. However, the R-IVIVCs developed with the slow and moderate formulations were unable to predict the R-enantiomer concentrations.

Table II presents the S-Cmax and S-AUC prediction errors for the racemate-IVIVC and the S-IVIVC. Correlations developed using the racemate-FRD vs racemate-FRA revealed a valid model when only the moderate and fast formulation were used (S-Cmax = 2.52%; S-AUC = 4.33%). Figures 5D–5F

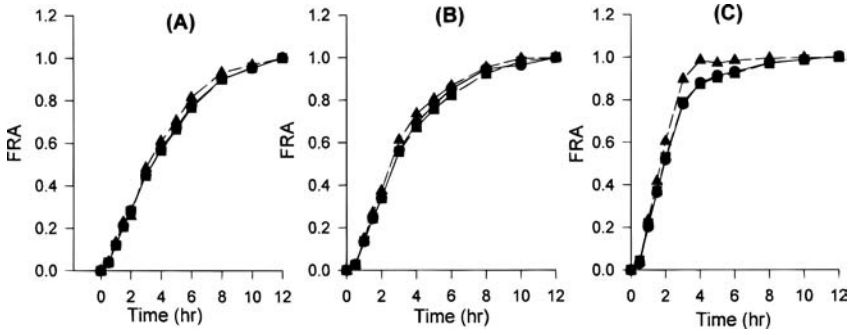

Fig. 3. Mean fraction of drug absorbed profiles for the racemate (●), S-metoprolol (■), and R-metoprolol (▲) after the administration of (A) slow, (B) moderate, and (C) fast formulations.

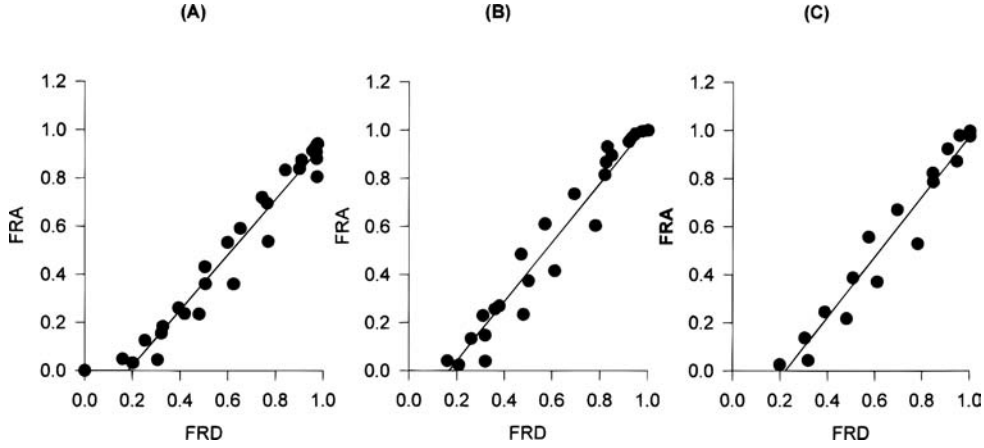

Fig. 4. S/M/F IVIVC model linear regression plots of FRA vs FRD for the (A) racemate, (B) R-metoprolol, and (C) S-metoprolol using Apparatus II, pH 1.2, 50 rpm.

present the observed and racemate-IVIVC model predicted S-metoprolol profiles for the correlation developed with the S/M/F formulation. Another racemate-IVIVC model (i.e., S/F) over- predicted the S-metoprolol Cmax, however this model was able to accurately predict the extent of drug absorption. These results suggest that the racemate drug can accurately predict the *in vivo* behavior of the active S-enantiomer.

When the S-IVIVC models (i.e., S/F, M/F, and S/M/F) were used to characterize the *in vivo* bioavailability of the S-enantiomer, they accurately predicted the rate as well as the extent of drug absorption for all models except the S/M (Cmax = 16.90%). Figures 6D–6F present the observed and S-IVIVC model predicted plasma S-metoprolol vs. time profiles for the correlation developed with the S/M/F formulation.

DISCUSSION

Bioavailability studies based solely on enantiomers provide pertinent information directly related to drug effect. Studies performed with racemic drugs without separate quantitation of enantiomeric disposition, may confound the pharmacokinetic properties of the active agent. This disparity is significantly compounded when the disposition of the enantiomers are not comparable. Pharmacokinetic differences between the R- and S- isomers of metoprolol have been well documented in the literature (3,4,15). Reports have shown a preferential first pass metabolism of the R-enantiomer as compared to S-metoprolol. Obviously, these differences have the potential to influence the development and predictability of an IVIVC with metoprolol racemate data.

The objective of this work was to examine the ability of an IVIVC developed with the racemic drug as well as the enantiomers, to predict the *in vivo* bioavailability of the enantiomers. Our results suggest that stereoisomerisms can significantly influence the validity of an IVIVC. The correlation developed with the racemate-FRD and racemate-FRA was not predictive of the *in vivo* bioavailability of the R-enantiomer (Table II). The averaged prediction errors of each of the racemate-IVIVCs (i.e., **S/M/F**, **M/F**, **S/M** and **S/F**) were greater than 38% for both R-Cmax and R-AUC. In evaluating the FRA profiles for the racemate and R-metoprolol, it was apparent that there were differences in the rate of drug absorbed. Considering the fast formulation, the racemate-FRA (Fig. 3C) reached the maximum absorption in about 8 hr while the FRA of R-enantiomer reached the maximum at 4 hr. These data suggested that the absorption characteristic of the racemate differs from that of R-enantiomer and that the racemate-FRA can not be used in place of the R-FRA. Therefore, the correlation between racemate-FRD vs racemate-FRA was not able to predict the R-behavior.

Conversely, the racemate-IVIVC which utilized the racemate-FRD and the racemate-FRA also, was predictive of the bioavailability of S-metoprolol after the slow, moderate and fast formulations. Prediction errors for these IVIVC models were less than 12% for all correlations except for model developed with the slow and moderate formulations. Nonetheless, it would appear that the racemate-IVIVCs are predictive of the S-metoprolol levels. One factor that supports this is that the fraction of total metoprolol absorbed is similar to the fraction

Table II. % Enantiomer Cmax and AUC Averaged Prediction Errors for the Racemate-IVIVC, R-IVIVC, and S-IVIVC Using USP Apparatus II, pH 1.2, 50 rpm.

IVIVC model formulations	Racemate-IVIVC				R-IVIVC		S-IVIVC	
	R-Cmax	R-AUC	S-Cmax	S-AUC	R-Cmax	R-AUC	S-Cmax	S-AUC
S/M/F	50.7	53.1	10.6	6.2	4.7	9.0	4.9	3.0
M/F	38.2	42.0	2.52	4.3	9.5	6.6	9.1	10.8
S/M	54.0	60.1	13.0	11.1	11.5	17.1	16.9	10.2
S/F	52.1	53.9	11.7	6.8	5.1	8.3	4.4	3.3

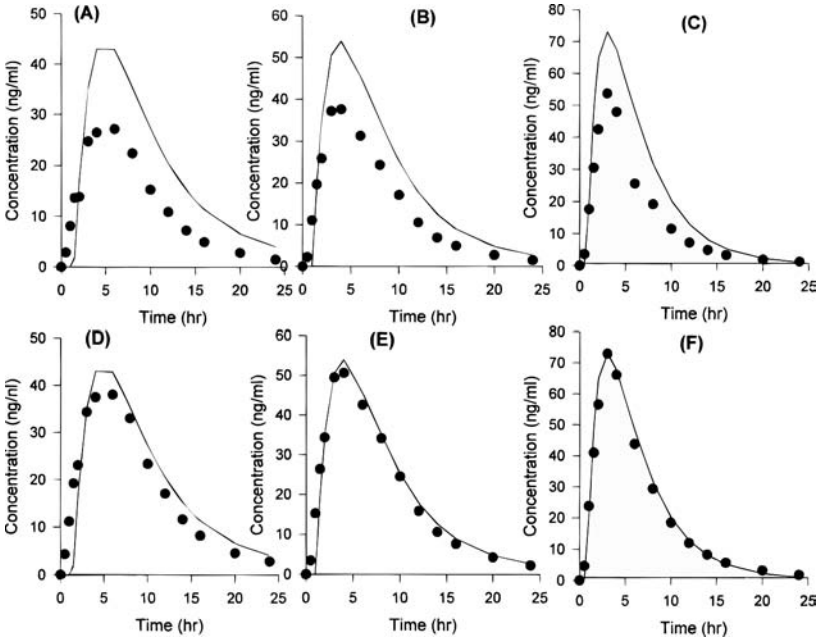

Fig. 5. Observed (●) and predicted (—) R-metoprolol plasma concentration for the (A) slow, (B) moderate, and (C) fast formulations using the slow/moderate/fast racemate—IVIVC model; and observed (●) and predicted (—) S-metoprolol plasma concentration for the (D) slow, (E) moderate, and (F) fast formulations using the slow/moderate/fast racemate-IVIVC.

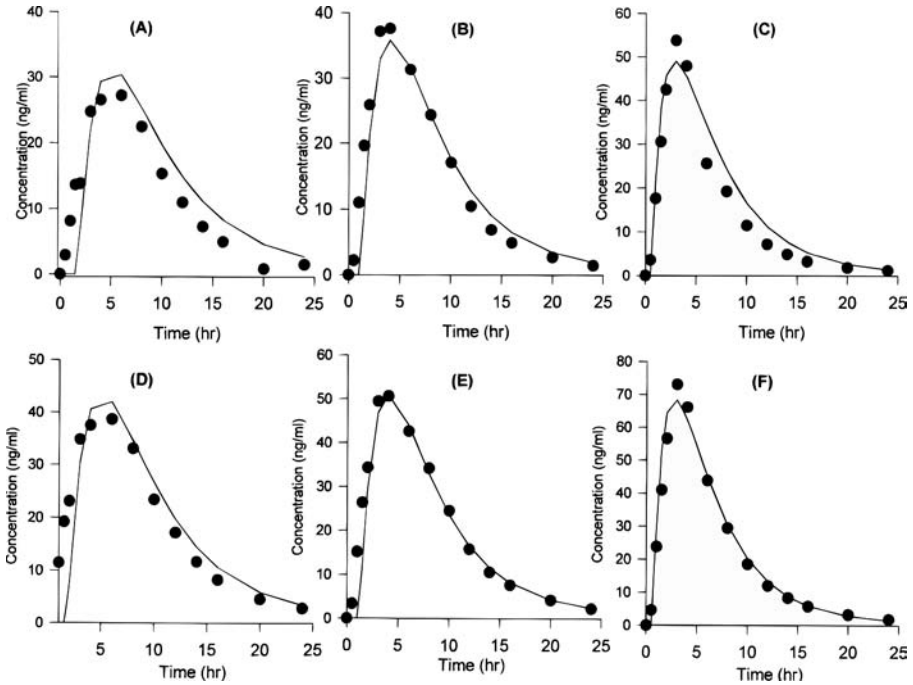

Fig. 6. Observed (●) and predicted (—) R-metoprolol plasma concentration for the (A) slow, (B) moderate, and (C) fast formulations using the slow/moderate/fast R-IVIVC model; and observed (●) and predicted (—) S-metoprolol plasma concentration for the (D) slow, (E) moderate, and (F) fast formulations using the slow/moderate/fast S-IVIVC model.

of S-enantiomer absorbed. Figures 3A–3C illustrate the similarity in fraction absorbed of the racemate and S-isomer for the fast, moderate, and slow release formulations. The fraction of drug absorbed in both profiles was almost identical for all release rates. This suggests that the racemic drug and S-metoprolol have similar absorption characteristics. In addition, metoprolol racemate and S-plasma profiles were also supportive of this finding. Similar absorption characteristics of the racemate and S-enantiomer are due to a negligible stereoselective first pass elimination of the S-enantiomer.

The correlations developed with R-FRD and R-FRA were in general accurate and predicted the *in vivo* bioavailability of the R-isomer. The prediction errors for each model (S/M/F, M/F, and S/F) were relatively low for both Cmax and AUC except for the IVIVC developed with the slow and moderate formulations. The relatively low prediction errors ($<10\%$) found for the bioavailability parameters strongly suggest that the R-metoprolol IVIVC models are valid and predictive of *in vivo* R-performance.

A more realistic measurement of the validation is the ability of a correlation to estimate the observed rate and extent of absorption for the active S-enantiomer. Correlations developed with S-FRD and S-FRA were found to be predictive of S-metoprolol plasma concentrations. The averaged %PE for Cmax and AUC across correlations was no more than 11% for IVIVCs developed with the S/M/F, M/F, and S/F formulations. However, again as seen with the R-IVIVCs, the correlation developed with the S/M formulations provided prediction errors outside the acceptable levels ($<17\%$). Nonetheless, based on the study results the correlation of S-FRD vs S-FRA provided a larger number of valid models as compared to the racemate in predicting the *in vivo* S-performance. It, therefore, would be reliable to use the S-dissolution and S-absorption data to develop the IVIVC for S-metoprolol.

The study was also conducted to examine whether the racemate data can be used to predict the *in vivo* R-enantiomer. It, therefore, may be concluded that the racemate data cannot be used in substitution of the R-enantiomer data in developing the R-IVIVC. Only the R-FRD and R-FRA are suitable for R-IVIVC development as supported by a large number of valid IVIVC models to be used as a true surrogate of R-bioavailability.

In summary, this research investigated the role of stereoisomerisms in the development and validation of an IVIVC using metoprolol as our model drug. Our results display that metoprolol racemate data cannot be used to accurately predict R-enantiomer drug concentration levels. However, the racemate data was predictive of the active stereoisomer. Further, enantiomer specific IVIVCs were predictive of *in vivo* performance. It should be noted that, even though the racemate data was predictive of the active enantiomer levels, caution should be used in applying the principles of IVIVC to racemic data. This work did display an inability of the racemate-IVIVC in predicting the drug levels of the inactive enantiomer. If the pharmacokinetic disposition significantly alters the plasma profile of an "active" enantiomer, a correlation developed with the racemate may provide misleading results. The assumption that an IVIVC developed with the racemate will accurately predict the plasma concentration profile of the active agent may not be valid.

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