

Figure 52: The individual QTcF values vs. d,l-nebivolol plasma concentrations for males (left panels) and females (right panels) on Day 1

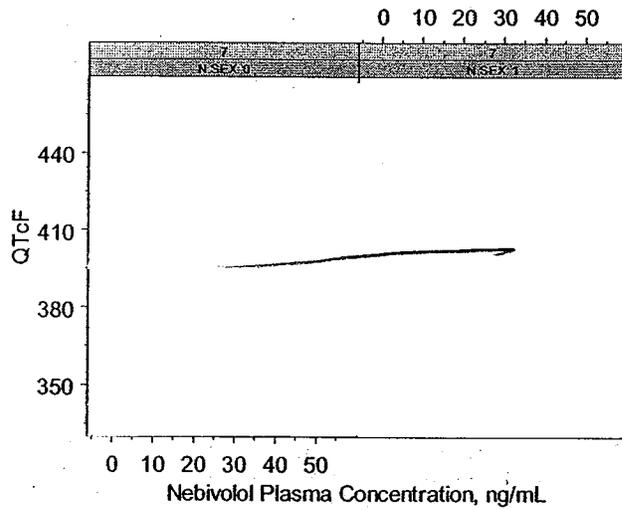


Figure 53: The individual QTcF values vs. d,l-nebivolol plasma concentrations for males (left panels) and females (right panels) on Day 7

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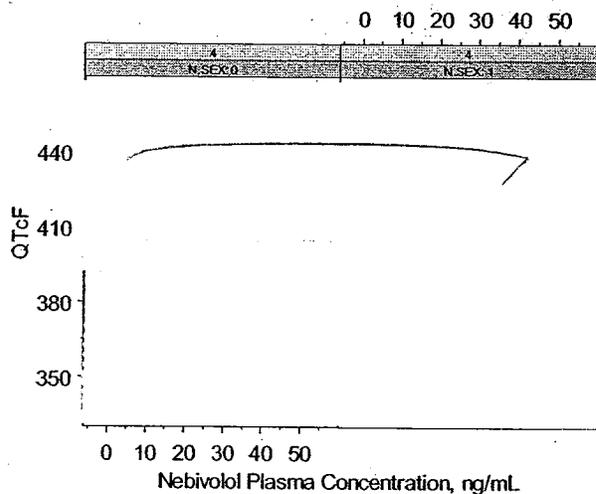


Figure 54: The individual QTcF values vs. d,l-nebivolol plasma concentrations for males (left panels) and females (right panels) on Day 4

The reviewer explored the relationship between changes in QTc calculated with Fredericia's formula and d,l-nebivolol plasma concentration. The individual QTcF versus plasma concentrations for EM subjects on Days 1, 4, and 7 are shown in Figure 41. Males are designated with SEX=0, and females with SEX=1. There is no evident trend in QTcF intervals related to the nebivolol plasma concentrations.

Figure 55 shows the d,l-nebivolol plasma concentrations vs. time on Day 7. The line is drawn at $\Delta QTc=0$ and the curve is the Loess smoothing line. The Loess line does not cross the line with $\Delta QTc=0$.

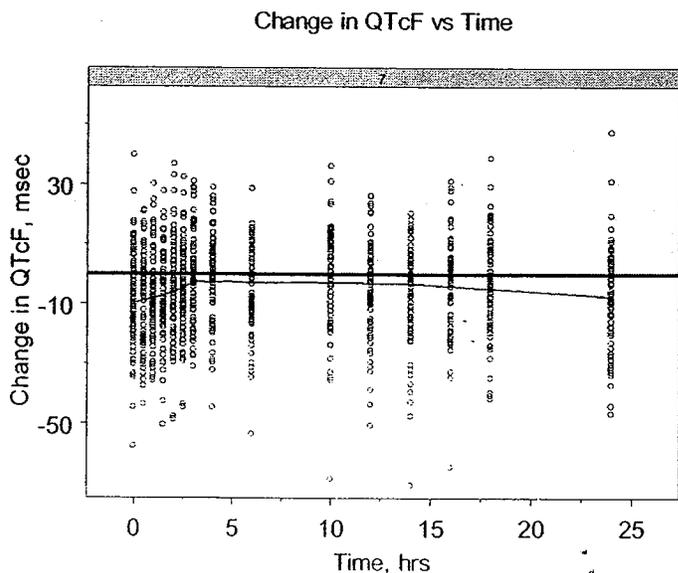


Figure 55: Changes in QTcF values vs. time post dose on Day 7. The curve is Loess smoothing line.

The sponsor reported that at 2 hours post-dose on Day 7, only one of the subjects had a QTcF interval larger than 450 msec. A total of 10 measurements of QTcF were larger than 450 msec but none was larger than 465 msec. None of the subjects had Δ QTc-F >60msec and only one subject #155 had Δ QTc-F of 50msec on Day 7 at 3 hours post-dose.

Table 84 lists the clinically notable QTc changes obtained on Day 7 at 2 hours post-dose.

Table 84: Subjects with Clinically Notable QTc-F Intervals or Increase from Baseline QTc-F Intervals at 2 Hours Post Dose on Day 7

QTc-F Signal Value	Nebivolol N=71	Atenolol N=60	Moxifloxacin N=67	Placebo N=69
n (%) of subjects with abnormal QTc-F				
≥ 450 msec	1 (1.4%)	2 (3.3%)	2 (3.0%)	0 (0.0%)
p-value*		0.593	0.611	1.000
≥ 480 msec	0	0	0	0
≥ 500 msec	0	0	0	0
≥ 30 msec increase from baseline	2 (2.8%)	2 (3.3%)	7 (10.4%)	1 (1.4%)
p-value*		1.000	0.090	1.000
≥ 60 msec increase from baseline	0	0	0	0

However, from 71 participated in the nebivolol group, twenty subjects on all days and nine subjects on Day 7 had an increase of QTc-F over 30msec.

For PM subjects who received nebivolol, (#0169, 141, 260), none had a change in QTc-F above 20 msec. Similar results were obtained in the atenolol group.

CONCLUSIONS:

1. The mean d,l-nebivolol C_{max} for EM subjects calculated by the sponsor on Day 1 (single dose of 20 mg), and Days 4, and 7 (daily doses of 40 mg) were 7.14, 15.57, and 20.76 ng/mL. In the previous study 0126, after a single dose of 20 mg nebivolol C_{max} was 4.64 ng/mL (d,l-nebivolol). The 54% higher value for C_{max} on Day 1 may be explained by the high inter-patient variability of nebivolol. With respect to C_{max}, the pharmacokinetics of d,l-nebivolol was linear after single doses from 2.5 to 20 mg. The pharmacokinetics of multiple doses of nebivolol above 10 mg was not studied previously. A two-fold change in C_{max} from Day 1 (20 mg dose) and Day 4 (40 mg dose after 4 doses of 20 mg) confirms the dose linearity for C_{max}. The 30% change in C_{max} from Day 4 to Day 7 may be attributed to the drug accumulation.
2. In the previous clinical studies, the sponsor demonstrated that nebivolol prolonged the QT interval. The increase in the QT interval was shown to be dose and heart rate dependent. When corrected for heart rate effects, the QTc interval tended to decrease with dose. The sponsor properly selected the correction method for the estimation of the QTc interval, since Bazett's formula undercorrect the QT at lower

heart rates Fridericia's formula generated more accurate QTc in subjects with extreme changes in heart rates.

3. The sponsor concluded that nebivolol does not prolong the QTc interval when administered chronically at the highest studied clinical dose of 40 mg daily. The conclusions were made by the comparison of nebivolol with the active control (atenolol), positive control (moxifloxacin) and placebo. All analyzed data compared the results at 2 hours post-dose on Day 7 with the baseline values. The reviewer performed an additional graphic data analysis to include all QTcF data. This analysis confirms that there is no relationship between the nebivolol plasma concentrations and QTcF. On average (Loess regression) the changes in QTcF were negative at any given time over the study. Therefore, the sponsor conclusion that nebivolol does not prolong the QTc interval is valid.

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4.2.13 A Double-Blind, Multi-Center, Randomized, Placebo-Controlled, Parallel Group Dosing Evaluating the Effects of Nebivolol on Blood Pressure in Patients with Mild to Moderate Hypertension (NEB- 302)

Investigational Product: Nebivolol Hydrochloride
Development Phase of Study: III
Study Dates: 19 Sep 2001 - 21 Mar 2003
Medical Officer: James H. Sherry, M. D., Ph. D. Medical Director

OBJECTIVES:

To determine if nebivolol is superior to placebo for the treatment of elevated blood pressure in patients with mild to moderate hypertension.

To determine the dose response relationship of nebivolol on blood pressure in patients with mild to moderate hypertension.

To compare the safety and efficacy of nebivolol in patients with mild to moderate hypertension.

STUDY DESIGN:

This study was a Phase III, multi-center, randomized, double-blind, parallel group, placebo-controlled study of nebivolol over a range of doses (1.25, 2.5, 5, 10, 20 and 30/40mg) in patients with mild to moderate hypertension. Patients were stratified across all treatment arms by the following factors in order of priority: metabolism of nebivolol (PM vs. EM); diabetes status (history of diabetes mellitus vs. no history of diabetes mellitus); race (Black vs. Non-Black); and age (< 65 and = 65) and gender. The study consisted of 2 phases: screening/washout/single-blind placebo run-in (28-42 days) followed by randomization/double-blind treatment (84 days). During the double-blind phase, patients received nebivolol or placebo. Patients randomized to nebivolol 40mg initially received nebivolol 30mg daily and after 2 weeks were titrated to 40mg once daily if their sitting heart rate was > 55bpm. Patients had 7 scheduled clinic visits during the study.

Number of Patients: 825 (75 placebo and 750 nebivolol)
Treatment: One tablet from each bottle once daily, orally.
Duration of Treatment: Up to 126 days (28-42 days placebo run-in; 84 days double-blind.)
Reference Therapy: Placebo tablets
Dose: Once daily oral
Administration: Treatment schedule identical to active therapy

Criteria for Evaluation:

The primary efficacy variable was the change of the average sitting diastolic blood pressure taken at trough drug plasma level (24 ± 2 hours post-previous morning's dose) at the end of treatment compared to baseline.

Dose titration only occurred with the 40mg treatment arm: patients randomized to this treatment arm began dosing of study medication at 30mg once daily and, if medically appropriate (sitting heart rate > 55bpm) after 2 weeks, up-titrated to 40mg once daily. Genomics testing were done on each patient to identify poor vs. extensive nebivolol metabolizers. A minimum of 7 clinic visits was conducted throughout the study. At each clinic visit blood pressure (supine, sitting, and standing) and heart rate (supine, sitting, and standing) were obtained before the morning dose (approximately 24 hours after the previous morning's dose time). Body weight was

Assay (Curve III)			
Linearity	0.04ng/mL to 3.0ng/mL		Satisfactory
Precision (CV %)	d-nebivolol ≤ 1.86	l-nebivolol ≤ 2.28	Satisfactory
Accuracy Between day	d-nebivolol between -4.09% and 6.24%	l-nebivolol between -4.77% and 4.51%	Satisfactory
LLOQ	0.04ng/mL		Satisfactory
Specificity			Satisfactory

Plasma was assayed also for non-conjugated plus conjugated nebivolol in human plasma (EDTA) using a high performance liquid chromatography with tandem mass spectrometric detection system. For total nebivolol (conjugate + non-conjugated nebivolol), the assay characteristics are show in Table 74.

Table 87: Assay for non-conjugated plus conjugated nebivolol

Parameter	Measure	Comment
Linearity	linear from 1.0ng/mL to 800ng/mL	Satisfactory
Precision (CV %)	≤ 5.7	Satisfactory
Accuracy Between day	between -3.12 and 3.11%	Satisfactory
LLOQ	1.0ng/mL	Satisfactory
Specificity		Satisfactory

4.2.14 Population Pharmacokinetic Data Analysis NEBI-302

The data analysis was performed by the sponsor.

OBJECTIVES

To apply a population pharmacokinetic model for d-nebivolol, l-nebivolol and nebivolol glucuronide(s) to the NEB- 302 trial in patients with mild to moderate hypertension.

To obtain estimates of typical pharmacokinetic parameters in the target population and of their inter- and intra- individual variability.

To evaluate the effects of patients' demographic characteristics, concomitant medications and other covariates on nebivolol pharmacokinetics.

DATA SOURCE

Sparse pharmacokinetic samples were collected from each patient during the Phase III trial. The nebivolol isomers (d- nebivolol and l- nebivolol) and nebivolol glucuronide(s) plasma samples were measured by a validated LC/MS method for pharmacokinetic analysis. A total of 2673 plasma samples collected from 734 patients were available for population PK analysis.

Nebivolol pharmacokinetic data obtained from 34 healthy male and female subjects (20 extensive metabolizers and 14 poor metabolizers) in two Phase I studies (NEBI- 0126 and NEBI- 0127) were used to develop the structural pharmacokinetic model.

SOFTWARE

NONMEM V level 1.1 () was used for all mixed-effect model fittings. The package was installed on a PC platform using Digital Visual Fortran 6.0a under MS Windows 2000. Data set preparation, exploration and visualization were performed using S-PLUS 2000 release 2 for Windows (Insightful, Seattle, WA, USA).

DATA PREPARATION

Measurements below the limit of quantification were excluded from the data sets. Log-transformed concentrations were used as the dependent variable in the population analysis. The individual records were excluded due to the lack of information on date and/or time of drug intake or plasma sampling and on the dose administered and/or due to the missing plasma concentration.

The covariates were included as follows:

- Gender (SEX, males = 0, females = 1)
- CYP2D6 Genotype (TYPE, EM = 0, PM = 1)
- Dose Group (DOSE, 1.25, 2.5, 5, 10, 20, 30 and 40mg)
- Race (RACE, Caucasian = 1, Black = 2, Hispanic = 3, Oriental = 4, Others = 5)
- Age (AGE), years
- Body weight (WT), kg
- Creatinine clearance (CRCL, mL/min), calculated as:
 - CRCL = $WT \cdot (140 - AGE) / 72 / CRT$ for males
 - CRCL = $0.85 \cdot WT \cdot (140 - AGE) / 72 / CRT$ for females
- Concomitant medications with the no. of patients > 15 (COM1- COMx, yes = 1, no = 0)

STRUCTURAL PK MODEL

The population pharmacokinetic model based on intensive sampling in two Phase I studies (NEBI- 0126 and NEBI- 0127) was used as a structural PK model. It was a two-compartment model with first-order absorption and lag time parameterized in terms of physiologic parameters. The interpatient variability was modeled with exponential error model in order to positively constrain individual parameter values, which were thus assumed to follow the lognormal distribution assumed to be in normal distribution. The residual variability in plasma concentrations was modeled using the additive error model (on logarithmic scale).

The empirical Bayesian estimates of individual random effects were obtained using the POSTHOC option. Random effects were plotted against covariates and analyzed using multiple regressions. Based on the visual examination of plots and multiple regressions the covariates were selected for the incorporation in the model. Continuous covariates were included using one of the following equations, depending on the nature of the effect:

$$TP = \Theta_n \cdot \left(\frac{Co\ variate}{MedianCo\ variate} \right)^{\Theta_{n+1}}$$

$$TP = \Theta_n \cdot \left(1 + \Theta_{n+1} \cdot \frac{Co\ variate - MedianCo\ variate}{MedianCo\ variate} \right)$$

The categorical covariates were described as follows:

$$TP = \Theta_n \cdot (1 + \Theta_{n+1} \cdot Co\ variate)$$

The covariate model was developed by stepwise forward addition and backward elimination. The covariate with the largest drop in the maximum likelihood objective function (MOF) was added to the model first. The likelihood ratio test was applied. For a covariate to be included, the drop in MOF should exceed 6.63 ($p < 0.01$, χ^2 , 1 df). A full model was determined when no additional improvement was possible. The final significance of each fixed effect was re-evaluated by deleting it from the full model. If the exclusion of a fixed effect results in the increase in MOF less than 7.88 ($p < 0.005$, χ^2 , 1 df) the covariate would be removed from the model.

The covariate model with non-correlated random effects (only the diagonal OMEGA matrix) was refined by testing correlations between random effects. If the correlation (OMEGA BLOCK) significantly improves the fit ($p < 0.001$), it was kept in the model. The covariance step was used in the final optimal model to obtain the estimates of standard errors of fixed and random effects.

The following diagnostics plots were generated and evaluated:

- Observed concentrations versus posterior population and individual predictions.

- Density plots of individual pharmacokinetic parameters

- Density plots for individual and population weighted residuals.

- Individual and population weighted residuals versus time post the latest intake.

- Individual and population weighted residuals versus individual and population predicted concentrations.

The optimal model was validated by a bootstrap technique. A minimum of 1000 replicates of the data were generated by bootstrap for NONMEM analysis to obtain the 95% confidence intervals (CI) of the fixed-effect and random-effect parameters. The non-parametric approach was used in which the NONMEM estimates were sorted by ascending order and the estimates at the 2.5% and 97.5% percentile were picked up as the lower and upper limits of the 95% CI.

RESULTS:

Pharmacokinetic Model for Healthy Subjects (rich data file)

The sponsor first fitted a two-compartment model with first-order absorption and lag time to 857 concentration-time data of *d*- and *l*-nebivolol and nebivolol glucuronides obtained from healthy male and female subjects after a single dose of 5, 10, or 20-mg tablet of nebivolol. Table below shows the disposition of the subjects within two studies and the number of plasma samples included in the data analysis.

Table 88: Subject' disposition

Study	Number of subjects ^a		Number of pharmacokinetic Samples ^b
	EM	PM	
NEBI-0126	6 M, 2 F	4 M, 3 F	572
NEBI-0127	8 M, 4 F	4 M, 3 F	285
Total	14 M, 6 F	8 M, 6 F	857

The parameters estimated for *d*-, *l*-nebivolol and nebivolol glucuronides are shown in the Table below.

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Table 89: Pharmacokinetic parameters in healthy subjects

Pharmacokinetic Parameter	Mean ^a		IIV (%)	IOV (%)
	EM (N=20)	PM (N=14)		
d-Nebivolol				
V2/F (L)	4260 (14.3)	1280 (38.0)	47.3 (70.5)	4.6 (876)
CL/F (L/h)	822 (23.4)	31.3 (48.9)	52.7 (101)	21.7 (74.8)
V3/F (L)	9940 (38.3)	251 (120)	69.8 (129)	35.8 (179)
Q/F (L/h)	2090 (25.9)	95.9 (195)	42.7 (290)	40.1 (152)
Ka' (1/h) ^b	1.86 (14.5)	1.18 (56.9)	160 (78)	116 (67.5)
Lag time (h)	0.2 fixed	0.2 fixed	44.6 (19.6)	-
l-Nebivolol				
V2/F (L)	2750 (25.7)	939 (79.3)	68.2 (189)	41.2 (100)
CL/F (L/h)	416 (28.6)	7.25 (46.5)	40.6 (189)	13.6 (47.5)
V3/F (L)	8410 (28.2)	366 (107)	47.4 (266)	34.9 (252)
Q/F (L/h)	1590 (25.3)	248 (165)	63.6 (181)	24.6 (283)
Ka' (1/h) ^b	3.0 (5.4)	0.58 (197)	252 (203)	130 (139)
Lag time (h)	0.2 fixed	0.2 fixed	4.9 (90.3)	3.1 (160)
Nebivolol Glucuronides				
V2/F (L)	212 (20.8)	66.2 (26.6)	37.9 (129)	17.1 (133)
CL/F (L/h)	37.6 (14.2)	3.9 (30.1)	29.4 (77.2)	17.1 (47.1)
V3/F (L)	69.6 (44.0)	75.2 (78.2)	46.6 (115)	17.9 (410)
Q/F (L/h)	7.78 (41.1)	7.78 (33.9)	45.2 (214)	11.7 (3096)
Ka' (1/h) ^b	1.79 (12.2)	1.50 (11.8)	275 (84.8)	98.8 (91.1)
Lag time (h)	0.22 fixed	0.15 fixed	37.4 (72.1)	30.4 (91.1)

Only genotype was included as a covariate in these models. The sponsor evaluated graphically the goodness of fits for each model. The sponsor concluded that all three models fitted the data satisfactorily.

The model proposed by the sponsor for nebivolol glucuronides assumed that the sum of these substances were available in plasma after oral dose of nebivolol the same way as if they would be administered individually orally. The first pass effect was not included in the model. Only the fraction of the parent drugs converted to the metabolite can be considered as an AMOUNT in the NONMEM routine and for the estimation of the clearance value. However, the fraction of conversion to the glucuronides was not estimated in the model. Moreover, the possible hydrolysis of the glucuronides into the parent compounds was not included in the model. In addition, studies NEBI-126 and NEBI-127, the pharmacokinetics of nebivolol glucuronides were poorly characterized for the low doses of nebivolol (see Comments to Individual Study Reviews of NEBI-126 and NEBI-127). The sponsor failed to measure the plasma concentrations of the nebivolol glucuronides for EMs (not enough assay sensitivity) and for PMs, the plasma concentrations of nebivolol glucuronides were not measured long enough to characterize the terminal phase of elimination. Therefore, the estimates of the AUC and clearance values deemed not acceptable. The sponsor recognized that in their study report, nevertheless, the results of the data analysis were presented in the report and were used for comparison with the estimates obtained by the population model.

In Figure 56 the sponsor showed the population predicted vs. observed and weighted residuals vs. population predicted plasma concentrations of d-, l-nebivolol and nebivolol glucuronides respectively.

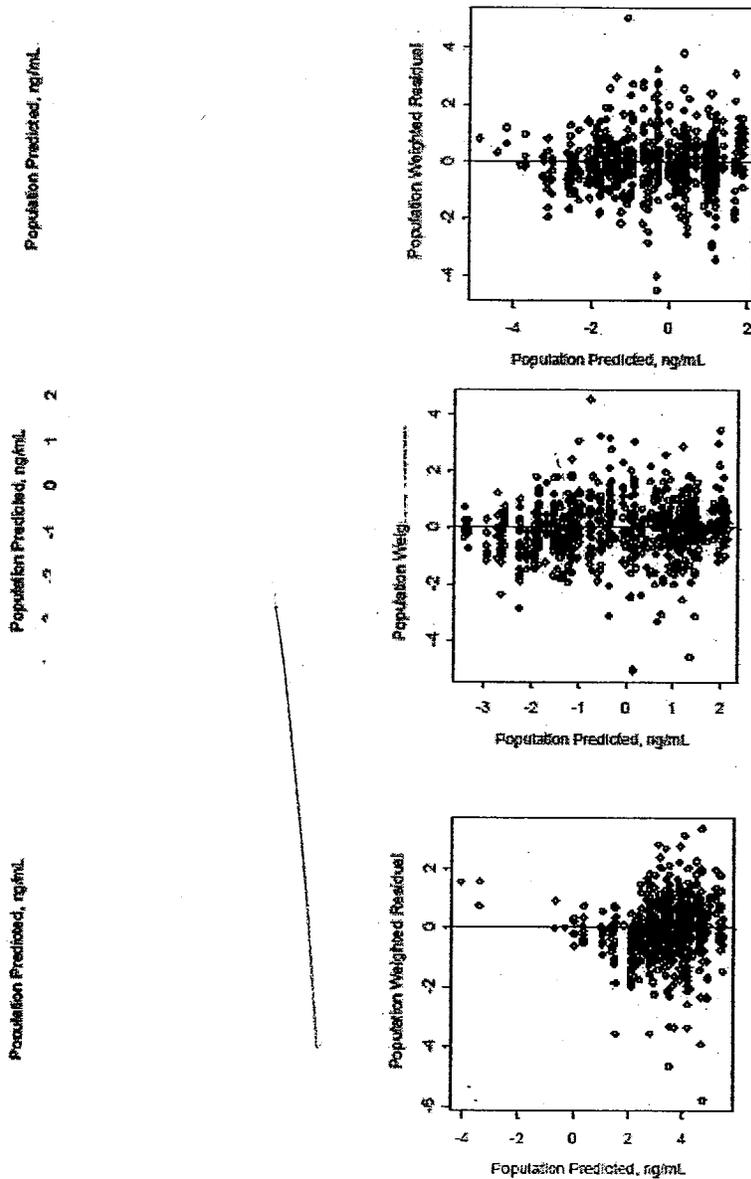


Figure 56: Model diagnostics for d-nebivolol (upper panel, l-nebivolol (middle panel) and nebivolol glucuronides (lower panel)

The prediction of the plasma concentration of the sum of the nebivolol glucuronides cannot be obtained using the population PK model. Treating the sum of nebivolol glucuronides as a single molecular entity is not reasonable from a modeling point of view. The oral clearance (CL/F) in EMs and PMs obtained from the models for d- and l-nebivolol were compared to those obtained

by noncompartmental method (Table below). The parameters estimated by the population model were about 20% smaller than the same parameters estimated by the non-compartmental model for both d- and l-nebivolol; however, the differences were not significant due to high intersubject variability.

Table 90: Comparison of the clearance (%CV) estimated using the population and non-compartmental approaches

CL/F estimates (L/hr) Mean ^a (%CV)	Extensive Metabolizers		Poor Metabolizers	
	NONMEM ^b	NCA ^c	NONMEM ^b	NCA ^c
d-Nebivolol	822 (52.7)	1041 (49.1)	31.3 (52.7)	38.8 (23.6)
l-Nebivolol	416 (40.6)	494 (45.5)	7.25 (40.6)	9.20 (22.8)
Nebivolol glucuronide(s)	37.6 (29.4)	41.7 (32.7)	3.9 (29.4)	5.1 (28.0)

^a Geometric mean was reported in NONMEM analysis, while arithmetic mean was reported in NCA analysis.

^b Same inter-subject variability in EM and PM subjects was assumed in NONMEM analysis.

^c CL/F estimated by non-compartmental method from NEBI-0127 study

In Table 90, the sponsor presented the clearance values estimated for nebivolol glucuronides. Note that clearance values estimated by the noncompartmental method were based on the AUC values. In studies NEBI-126 and NEBI-127 nebivolol glucuronides were not measured in plasma of PMs long enough to properly calculate the terminal half-life and in EMs the measurements were limited at low doses.

Pharmacokinetic Population Model for the Patients (sparse data file)

The population pharmacokinetic models for d- and l- nebivolol and nebivolol glucuronides were fitted to data from study NEB- 302. In these PK models, the sponsor fixed almost all pharmacokinetic parameters to the values obtained from the healthy volunteer's data. Only clearance and volume of distribution of the central compartment were estimated. The effect of the covariates was evaluated only for oral clearance. The sponsor tested the following covariates: gender, race, age, smoking status, diabetic status, nebivolol dose level, creatinine clearance, body weight and concomitant medications.

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Table 91: Demographic Summary for Patients Included in the NONMEM Analysis

COVARIATES*	All	EM	PM
Gender:			
Male	418	390	28
Female	316	295	21
Age:			
Age > 65 yr	140	130	10
Age < 65 yr	594	555	39
Race:			
Caucasian	525	485	40
Black	98	96	2
Hispanic	98	92	6
Oriental	6	6	-
Smoker:	151	137	14
Diabetic:	67	66	1
Weight, kg:	85.6	85.8	82.7
(Range)	(47-130)	(47-130)	(52-114)
Creatinine Clearance, ml/min (Range)	117 (30-316)	117 (30-316)	120 (55-240)

Table 92: Summary of Clinical Laboratory Data by CYP2D6 Genotype for Patients Included in the NONMEM Analysis

Variable ^a		EM	PM	All
Scr (mg/dL)	N	683	49	732
Normal Range:	Mean	0.9	0.8	0.9
0.5 - 1.4	(Range)	(0.4 - 1.9)	(0.4 - 1.2)	(0.4 - 1.9)
CLcr (ml/min)	N	682	49	731
	Mean	116.7	119.5	116.9
	(Range)	(29.8 - 316)	(55.2 - 240)	(29.8 - 316)
ALT (U/L)	N	683	49	732
Normal Range:	Mean	23.7	24.1	23.7
0 - 48	(Range)	(3.0 - 98.0)	(7.0 - 112)	(3.0 - 112)
AST (U/L)	N	683	49	732
Normal Range:	Mean	23.0	22.0	22.9
0 - 55	(Range)	(8.0 - 136)	(14.0 - 50.0)	(8.0 - 136)
Total bilirubine (mg/dL)	N	683	49	732
Normal Range:	Mean	0.6	0.6	0.6
0.1 - 1.3	(Range)	(0.1 - 3.4)	(0.2 - 1.5)	(0.1 - 3.4)
Alkaline phosphatase (U/L)	N	683	49	732
Normal Range:	Mean	77.6	75.8	77.5
20 - 125	(Range)	(24.0 - 219)	(43.0 - 118)	(24.0 - 219)

Table 92 shows the clinical laboratory test results obtained from the patients included in NONMEM data analysis. The sponsor concluded that there was no apparent difference between EM and PM patients. Mean values for all parameters were similar, however, the upper range for the EM subject' parameters were above normal indicating that some patients had renal impairment and/or hepatic abnormalities. Nevertheless, the covariates related to the hepatic function were not tested by the sponsor.

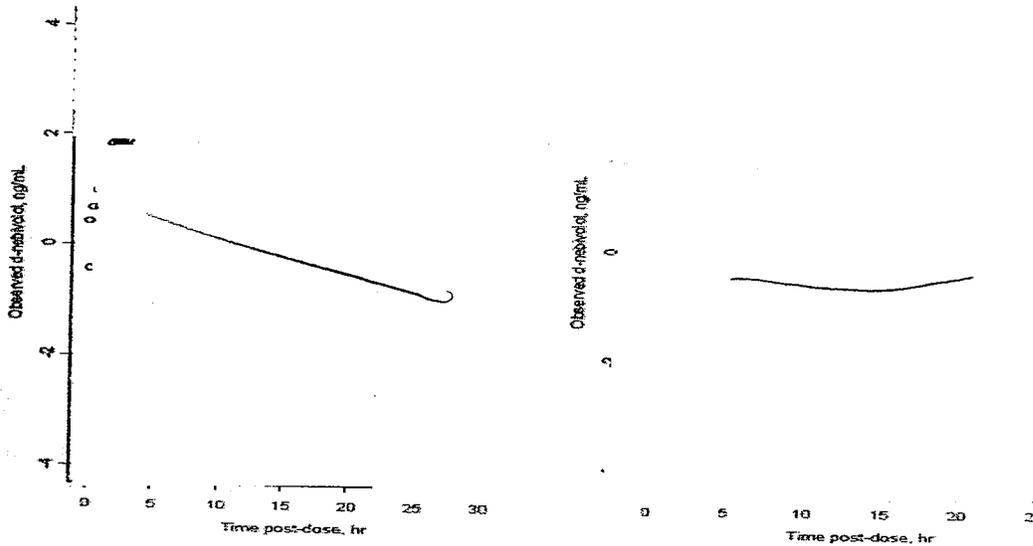
Concomitant medications taken by less than 15 patients were not included in the analysis.

Table 93: Summary of Concomitant Medications by CYP2D6 Genotype for Patients Included in the NONMEM Analysis

Concomitant Medications	EM	PM	All
ACETYLSALICYLIC ACID	111 (16.2%)	9 (18.4%)	120 (16.3%)
PARACETAMOL	68 (9.9%)	5 (10.2%)	73 (9.9%)
ATORVASTATIN	55 (8.0%)	3 (6.1%)	58 (7.9%)
TOCOPHEROL	48 (7.0%)	3 (6.1%)	51 (6.9%)
IBUPROFEN	33 (4.8%)	1 (2.0%)	34 (4.6%)
SIMVASTATIN	27 (3.9%)	2 (4.1%)	29 (4.0%)
LEVOTHYROXINE SODIUM	26 (3.8%)	-	26 (3.5%)
METFORMIN	21 (3.1%)	-	21 (2.9%)
ESOMEPRAZOLE	16 (2.3%)	2 (4.1%)	18 (2.5%)
SILDENAFIL CITRATE	16 (2.3%)	-	16 (2.2%)

Table 93 shows the concomitant medications which were used in patients included in NONMEM data analysis.

Figure below shows the observed plasma concentrations for d-, l-nebivolol and nebivolol glucuronides



d- Nebivolol

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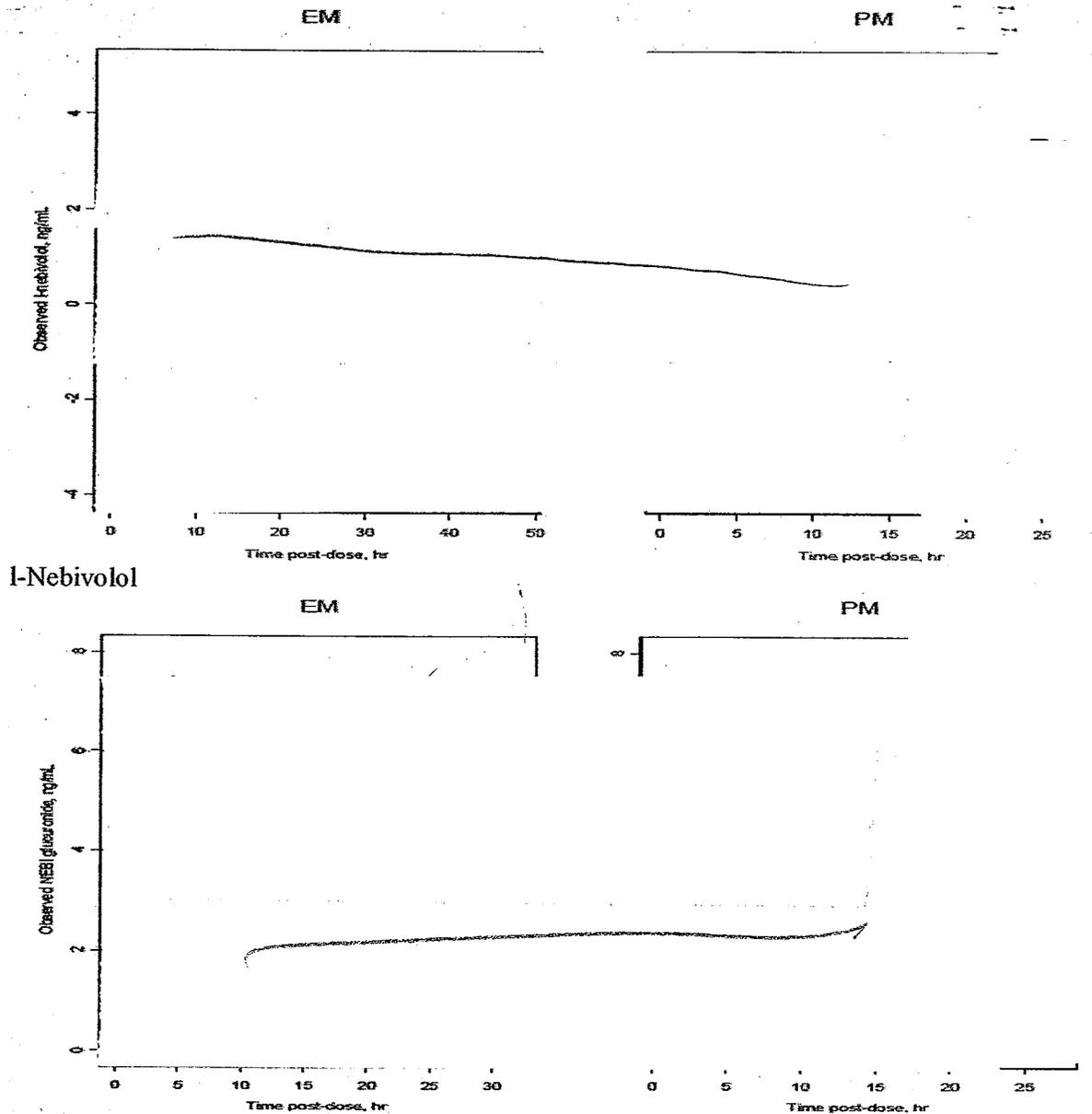


Figure 57: Plasma concentrations vs. time

The plasma sampling in this study was poorly designed. Although the number of samples (3-4 per subject) was sufficient, the sampling occurred only at the peak and trough plasma concentrations and there was no information about the plasma concentration profiles in between of these points. The Population PK Guidance for the industry recommends to have 3-4 plasma samples per patients which are obtained in a few intervals to proper characterize the plasma concentration vs. time profile.

Covariate effects on CL/F of EMs and PMs were evaluated separately. The addition of the effect of creatinine clearance on CL/F of *d*- and *l*-nebivolol, and glucuronides in EM patients was significant. It was also found to be significant for CL/F of *l*-nebivolol in PM patients. The

creatinine clearance effect on CL/F of d-nebivolol and glucuronide in PM patients was insignificant may be due to the small sample size. Other covariate effects on CL/F of *d*- and *l*-nebivolol, and glucuronides in PM patients were not tested due to the small sample size. The addition of the inter-occasional variability (IOV, visits 5 and 7) significantly improved the fit for *d*- and *l*-nebivolol. The IOV were estimated using the SAME option assuming the variance of IOV to be the same as IIV. The effect of race was not formally tested in model building step. The sponsor evaluated graphically the effect of four race categories on the Bayesian estimates of CL/F and did not find the obvious difference in CL/F. The covariate effects on the volume of distribution of the central compartment were not evaluated.

The pharmacokinetic parameters estimated for d-nebivolol are shown in the Table below.

Table 94: PK Parameters of d-nebivolol

Pharmacokinetic Parameter	Central Tendency ^a		IIV (%) ^b
	EM (N=685)	PM (N=49)	
V ₂ /F (L)	3720 (4.4)	1850 (22.5)	87.3 (66.1)
CL/F (L/h)	635 (3.1)	48.8 (12.7)	47.2 (8.4)
Effect of CLcr on CL/F	0.29 (25.5)	-	-

The evaluation of goodness of fit is shown in Figure 58.

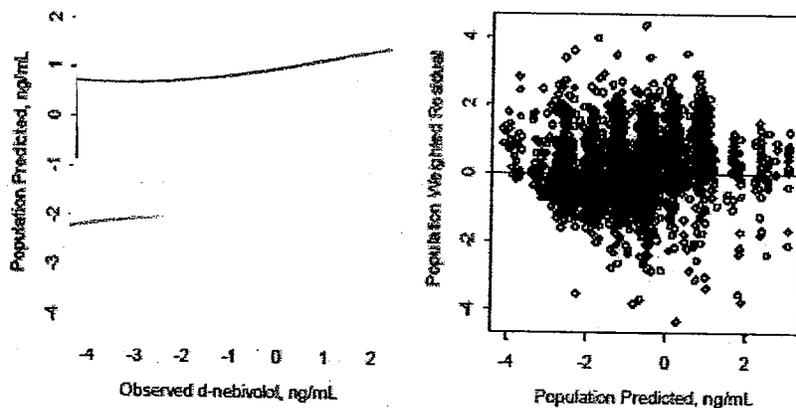


Figure 58: Diagnostics plots for d-nebivolol

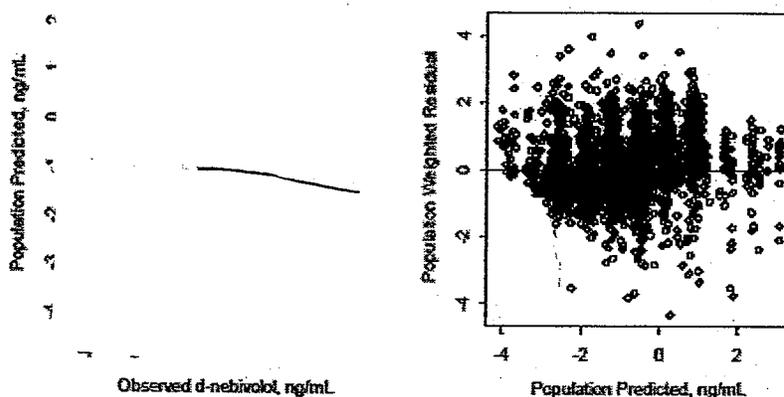
The parameters for the optimal l-nebivolol pharmacokinetic model are shown in Table below.

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Table 95: PK parameters of l-nebivolol

Pharmacokinetic Parameter	Central Tendency ^a		IIV (%) ^b
	EM (N=685)	PM (N=49)	
V ₂ /F (L)	2920 (4.1)	867 fixed	127 (36.7)
CL/F (L/h)	413 (2.9)	17.8 (23.8)	47.3 (10.6)
Effect of CL _{cr} on CL/F	0.215 (28.0)	0.655 (103)	

Figure below demonstrates the goodness of fit for l-nebivolol.

**Figure 59: Diagnostics plots for l-nebivolol**

The sponsor used the bootstrap technique to validate the models for d-, l-nebivolol and nebivolol glucuronide. These validations are acceptable for d- and l-nebivolol.

The use of compartmental modeling to describe the pharmacokinetics of the sum of nebivolol glucuronides as it was a single molecular entity does not have any physiologic meaning. The parameters estimated by the sponsor for nebivolol glucuronides are not acceptable.

Discussion of the Covariate Effects

The sponsor was only able to detect the significant covariate effect of creatinine clearance on oral clearance. The most important effect could be in the African-American group of patients. The reviewer compared the clearance values estimated for different races using box plots. In EM subjects, there is no apparent change in clearance for any of the studied races. However, the median clearance values for the PM subjects were 2-3 times larger for the Blacks compared to Whites both for d- and l-nebivolol. The race effect is shown in Figures below (Figure 60-Figure 63).

It is difficult if not impossible to quantify the effect of race in this study because the signal may be diffused: Blacks were represented by only 18% of the analyzed population and there were totally only 49 PMs in this study.

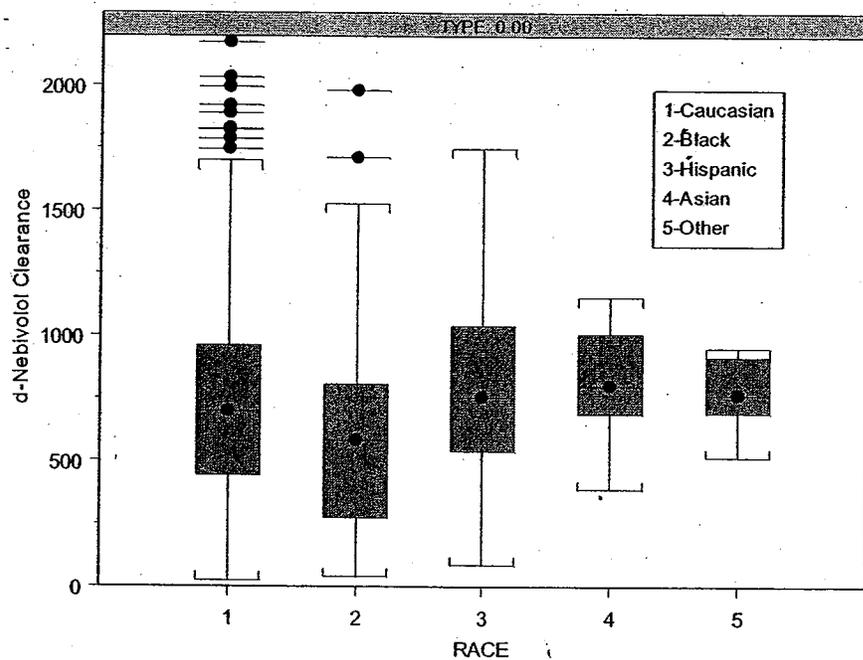


Figure 60: d-Nebivolol Clearance vs. Race EM subjects

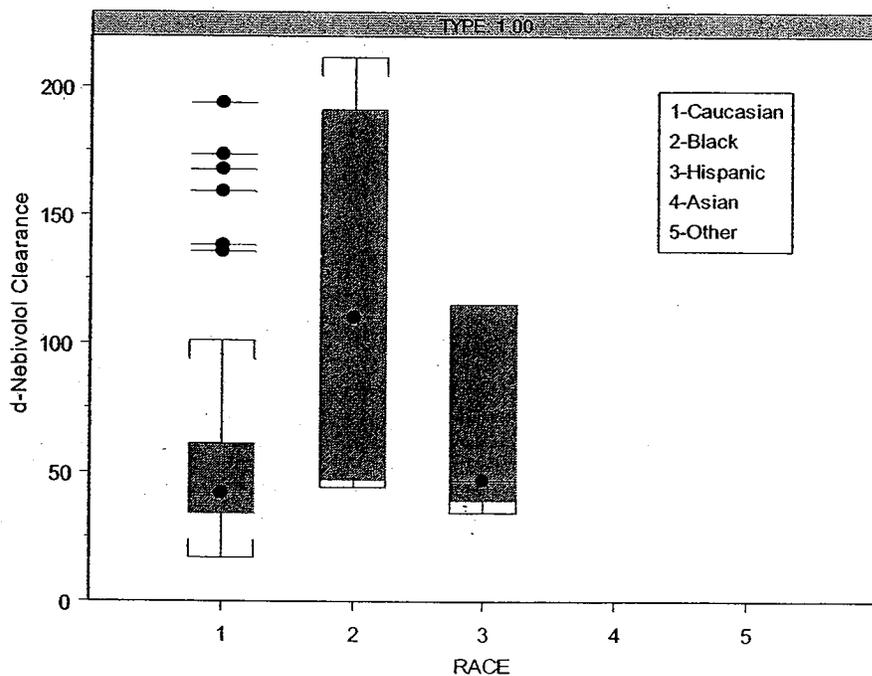


Figure 61: d-Nebivolol Clearance vs. Race, PM subjects

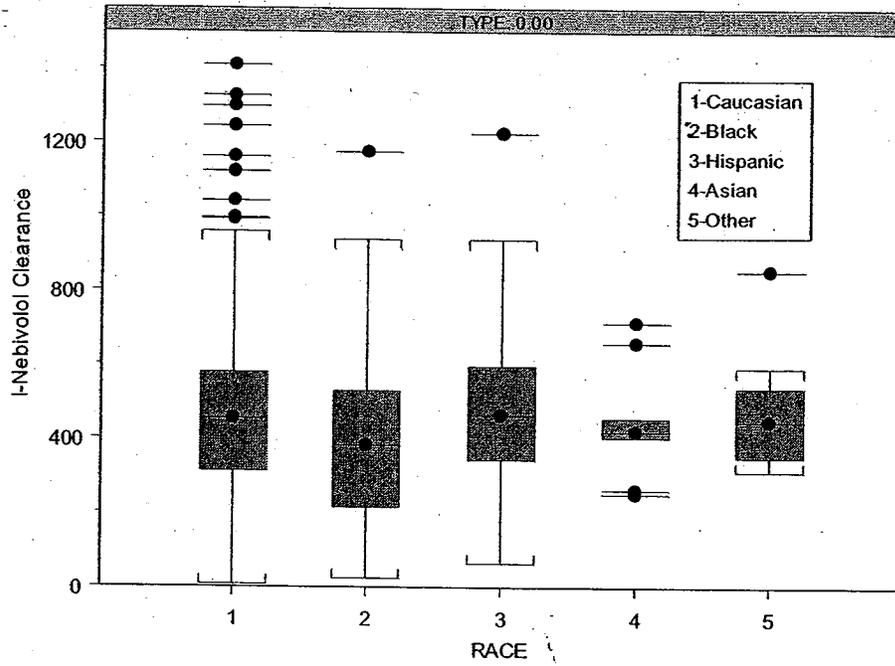


Figure 62: Clearance of l-nebivolol in EM subjects

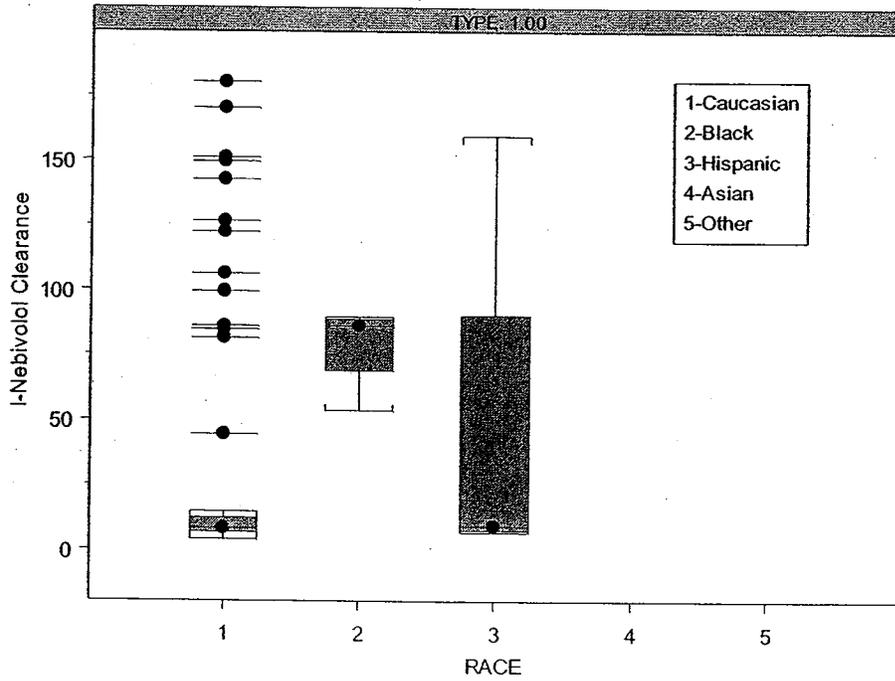


Figure 63: Clearance of l-nebivolol in PM subjects

All other covariates did not show any significant trends according to the graphic data analysis.

COMMENTS

1. The plasma sampling in this study was poorly designed. Although the number of samples (3-4 per subject) was sufficient, the sampling occurred only at the peak and trough plasma concentrations and there was no information about the plasma concentration profiles in between of these points. The Population PK Guidance for the industry recommends having 3-4 plasma samples per patients which are obtained in a few intervals to properly characterize the plasma concentration vs. time profile.
2. The pharmacokinetics of d- and l-nebivolol was previously described in studies NEBI-126 and NEBI-127. In these studies, the limitation of the assay for the low doses of drug and failure to obtain plasma samples at least up to 3 half-lives led to poor characterization of the nebivolol pharmacokinetics particularly for the low doses and for the PM subjects. Nevertheless, the parameters estimated in these studies by the non-compartmental method were used by the sponsor as a reference to the acceptance of the population model estimation. Moreover, all parameters (except for clearance (CL) and volume of distribution of the central compartment, (Vd)) obtained in healthy subjects were fixed for the patient population data analysis in order to estimate the patient's CL and V. The sponsor assumed that the pharmacokinetics of d- and l-nebivolol in healthy subjects and patients were similar but this assumption was never tested. Although the pharmacokinetic parameters estimated for d- and l-nebivolol were cited as "comparable" for the healthy and patient population, the clearance in the patient population was reduced for d-nebivolol by 20%, EMs and 55% PMs; and for l-nebivolol, no change for EMs and increased 2.5 times for PMs).
3. The sponsor should consider using the healthy subject's pharmacokinetic parameters as initial estimates in the development of the population pharmacokinetic model for the patients.
4. Mean values for all laboratory tests were similar, however, the upper range for the EM subject' parameters were above normal indicating that some patients had renal impairment and/or hepatic abnormalities. Nevertheless, the covariates related to the hepatic function were not tested by the sponsor.
5. The sponsor was only able to detect the significant covariate effect of creatinine clearance on oral clearance. The most important effect could be in the African-American group of patients. The reviewer compared the clearance values estimated for different races using box plots. In EM subjects, there is no apparent change in clearance for any of the studied races. However, the median clearance values for the PM subjects were 2-3 times larger for the Blacks compared to Whites both for d- and l-nebivolol. It is difficult if not impossible to quantify the effect of race in this study because the signal may be diffused: Blacks were represented by only 18% of the analyzed population and there were only 49 PMs included in this study. Therefore, an additional study is needed to evaluate the effect of race as a covariate on the pharmacokinetics and the pharmacodynamics of nebivolol.

6. In general, the population model described the pharmacokinetics of *d*- and *l*-neбиволol with a lot of assumptions and particularly was based on the parameters obtained for the healthy subjects. Due to poor study design, the pharmacokinetic profiles were not properly characterized and the effects of the important covariates were not quantifiable.
7. In studies NEBI-126 and NEBI-127, neбиволol glucuronides were inadequately characterized for the low doses of neбиволol (2.5 and 5 mg, see Comments to Individual Study Reviews of NEBI-126 and NEBI-127). The sponsor failed to measure the plasma concentrations of the neбиволol glucuronides for EMs (not enough assay sensitivity) and for PMs, the plasma concentrations of neбиволol glucuronides were not measured long enough to characterize the terminal phase of elimination. Therefore, the estimations of the AUC values and clearance values were deemed not acceptable. The sponsor recognized that in their study report, however, the results of the data analysis were presented in the report. Nevertheless, the parameters estimated based on these data were used for the comparison with the parameters estimated for the patients in study NEB-302.
8. More complicated models may be required to account for extensive first-pass metabolism and deconjugation of the glucuronides to form *d*-, and *l*-neбиволol. The model proposed by the sponsor for neбиволol glucuronides assumed that the sum of these substances were available in plasma after oral dosing of neбиволol the same way as if they would be administered individually orally. The first pass effect was not included in the model. Moreover, the possible hydrolysis of glucuronides into the parent compounds was not included in the model. The population model for neбиволol glucuronides is not acceptable.

4.2.15 Pharmacokinetic/Pharmacodynamic Modeling NEBI-302

Objectives:

To explore the relationship between plasma *d*, *l*-neбиволol concentrations (sum of *d*- and *l*-neбиволol at peak and trough) and the corresponding primary efficacy measurements in a mixed-effect PKPD model and to identify any potential covariate effects on the PKPD relationship.

Data

There were 3944 PK/PD measurements from 829 evaluable patients with both pharmacodynamic and pharmacokinetic measurements including 336 PK/PD measurements from 69 patients in the placebo group.

PK/PD Models for Blood Pressure and Heart Rate Responses

The pharmacodynamic/pharmacokinetic data for PD modeling included the averaged sitting diastolic and systolic blood pressure and heart rate measured three times at the trough and peak in visits 3, 5 and 7 and the corresponding plasma *d*, *l*-neбиволol concentrations (sum of *d*- and *l*-neбиволol at peak and trough). The sponsor assumed that since the contribution of *d*-neбиволol, *l*-neбиволol and neбиволol glucuronides to efficacy measurements is unknown and their concentrations are highly correlated, only the sum of *d*- and *l*-neбиволol peak and trough concentrations will be used in the PKPD modeling.

A mixed effect PD model was used to describe the PD response (g) as follows:

$$g = \text{Baseline} - E_0 + f_d(C)$$

the placebo effect was described:

$$E_0 = B_1 \cdot (1 - F_1) + B_2 \cdot F_1$$

peak was set to 0 for the trough and 1 for the peak measurements.
Drug effect, f_d was characterized by an Emax model as follows:

$$f_d(C) = \frac{E_{\max} \cdot C}{(EC_{50} + C)}$$

where Emax is the maximal drug effect, EC50 is the drug concentration that can produce 50 % of the maximal effect.

The inter-individual variability in baseline, E_0 , Emax and EC50 were modeled with an exponential error model. The residual variability in PD measurements was modeled with an additive error model assumed to follow the normal distribution.

The model was built in three sequential steps. In the first step, only the placebo data was used to build the placebo model. Any potential effects of covariates were evaluated in the placebo model. In the second step, data from placebo and active drug treatment groups were combined to build the drug effect model with fixed placebo parameters (B_1 and B_2) estimated from the first step. The baseline was estimated only from the baseline data in these two steps. In the third step, the PD model with non-correlated random effects (only the diagonal OMEGA matrix) was refined by testing correlations between random effects. The covariance step was implemented in the final optimal model to obtain the estimates of standard errors of fixed and random effects. Diagnostics plots of the final model were provided.

The following covariates were included in the analysis: gender, race group (African American and non-African American), age group (Elderly, ≥ 65 years old and Young, < 65 years old), smoking status, diabetic status, body mass index ($BMI \geq 30$, and $BMI < 30$). The demographic summary of patients included in the pharmacodynamic analysis is presented in Table 96.

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Table 96: Demographics of Patients Included in the Pharmacodynamic Analysis

Variable	Category	N (%)
Gender	Male	467 (56.3%)
	Female	362 (43.7%)
Race	Caucasia	588 (70.9%)
	Black	117 (14.1%)
	Hispanic	110 (13.3%)
	Oriental	6 (0.7%)
	Other	8 (1.0%)
Age Group	Elderly, ≥ 65 yr	153 (18.5%)
	Young, < 65 yr	676 (81.5%)
Smoker		170 (20.5%)
Diabetics		77 (9.3%)
BMI	< 30	419 (50.5%)
	≥ 30	410 (49.5%)
CYP2D6 Genotype	EM	773 (93.2%)
	PM	56 (6.8%)

Model Development

The sponsor proposed a saturable Emax drug effect model for the averaged sitting diastolic blood pressure (DSBP) and heart rate (HR) measurements. The attempt to model the sitting systolic blood pressure vs. nebivolol plasma concentration failed. The sponsor claimed that the measurements were too variable to reliably estimate the parameters.

First, the placebo model for DSBP was built based on the placebo data only. The potential covariate effects were then tested in the placebo model, and none of them was found significant. The data from the placebo arm and treatment arms were then combined, the placebo effect parameters were fixed and the drug effect model was developed. The effect of the covariates on Emax and EC50 was tested but none of them was found to be significant. The final model included the correlation between E0 and EMAX (OMEGA BLOCK function). The diagnostics plots are shown in Figure 64 and Figure 65.

Although the individual fits were acceptable, the population predicted vs. observed DBP were not distributed equally around the line of identity, and the weighted residuals vs. observed DBP plot was skewed, where at low blood pressure the model overpredicted the response and at high DBP the response was underpredicted.

These model diagnostic plots indicate that the model fit was unacceptable.

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Nevertheless, the sponsor estimated parameters for DSBP model, Table below.

Table 97: PD Parameter Estimates for Sitting Diastolic Blood Pressure

Parameter	Estimate	SE of Estimate
Baseline (mm Hg)	99.3	0.2
E_{max} (mm Hg)	-5.68	0.34
EC_{50} (ng/mL)	0.068	0.016
B_1 (mm Hg)	-5.26 fixed	-
B_2 (mm Hg)	-7.16 fixed	-
Variance of Baseline	0.00032	0.00008
Variance of E_0	1.01	0.21
Variance of E_{max}	1.11	0.34
Variance of EC_{50}	3.36	1.72
Covariance of Baseline and E_0	-0.015	0.002
Covariance of E_{max} and E_0	-0.62	0.21
Residual variance	20.0	0.4

Similar model building technique was used to evaluate the relationship between heart rate and the sum of d- and l-nebivolol plasma concentrations. The same problems with the population model fit were obvious in the heart rate model.

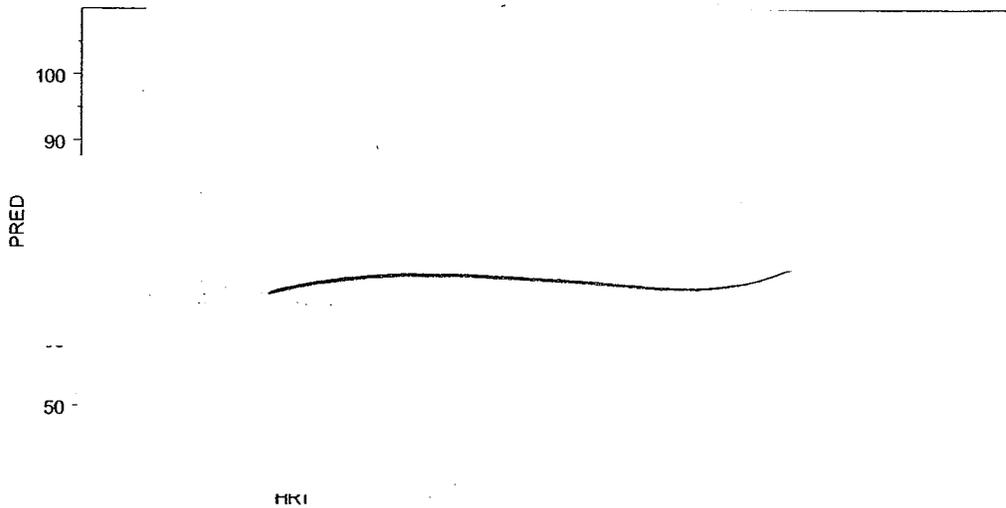


Figure 66: Population predicted vs. observed (left) and WRES vs. observed HR

The parameters estimated by the sponsor for this model are shown in Table below.

Table 98: PD Parameter Estimates for Sitting Heart Rate

Parameter	Estimate	SE of Estimate
Baseline (bpm)	72.1	0.3
E _{max} (bpm)	-6.84	0.29
EC ₅₀ (ng/mL)	0.016	0.006
B ₁ (bpm)	0 fixed	-
B ₂ (bpm)	0 fixed	-
Variance of Baseline	0.011	0.001
Variance of E _e	23.7	4.4
Variance of E _{max}	0.514	0.15
Variance of EC ₅₀	<0.0001	<0.0001
Covariance of Baseline and E ₀	-0.21	0.04
Covariance of E _{max} and E ₀	1.76	0.67
Residual variance	23.7	0.5

The sponsor did not make any conclusions which would relate the estimated PD parameters to the known physiologic factors.

COMMENTS:

1. It is well recognized that for the E_{max} saturable model, the estimation of EC₅₀ (50% of the drug concentration responsible for the maximal effect) should correlate with the receptor activity measured by K_i. The most comprehensive assessment of K_i of nebivolol in human myocardium is described in Maack et al 2001. In this paper the authors estimate the pK_i of β₁-adrenoceptor as about 7-8 mol/L which reflects the concentration at the effect site of 5-15 ng/mL. The effect site is heart; therefore, d-nebivolol (the isomer with the preferential β₁-adrenoceptor activity) plasma concentrations would be the closest approximation of the drug concentration which drives the effect. The sponsor's estimation of EC₅₀ for the sitting diastolic blood pressure was 0.068 ng/mL. This value is 220 fold higher than K_i. Moreover, the average d-nebivolol plasma concentrations measured in Study NEBI-302 was about 6 ng/mL, this value was the same order of magnitude as the K_i value. In this study, the effect of lowering blood pressure was achieved. The EC₅₀ values estimated by the sponsor do not reflect the physiologic parameters (K_i) for β-adrenoceptor activity of nebivolol.
2. The data available in this study did not allow to evaluate if there is a lag time between the pharmacokinetics and pharmacodynamics of the drug. The model proposed by the sponsor is not able to rule this out. The hysteresis could only be assessed if the full plasma concentration vs. time profile with the corresponding PD measurements would be available. Based on all of the above, the PK/PD model proposed by the sponsor is not acceptable. The attempt to describe the data with a linear PK/PD model (FDA reviewer) did not lead to a better model fit.
3. The EC₅₀ value for heart rate was estimated by the sponsor as 0.016 ng/mL. Same comments as above for DBP is applicable for the heart rate response model.

4. The pharmacokinetic plasma sampling for this study was not properly designed. Moreover, the assumption that the effect of the drug correlates with the sum of d- and l-nebivolol plasma concentrations is not reliable. It is possible that these factors led to the difficulties to obtain the reasonable parameter estimates for the Emax model.
5. The model diagnostics plot indicate that the population predictions for the diastolic blood pressure and heart rate do not correlate with the observed values, therefore, the models are not acceptable.
6. The implications of PK/PD modeling do not affect the labeling of nebivolol, therefore the information about the population modeling should be excluded from the label.

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4.2.17 Assay Information Relevant to Drug-Drug Interaction Evaluations

The applicant reported pharmacokinetic measures for the following entities that were obtained from plasma concentration time data directly (d- and l-nebivolol) or indirectly [d,l-nebivolol, and nebivolol glucuronides (G-UD)]. The applicant added individual plasma concentrations of d-nebivolol and l-nebivolol for each subject to provide an estimate of the d,l-nebivolol plasma concentration. Subsequently, the individual plasma concentration for d,l-nebivolol was subtracted from the corresponding conjugated plus non-conjugated nebivolol (total) plasma concentration to obtain the individual G-UD plasma concentration.

Reviewer Comment on Assays for total nebivolol and G-UD

The validity of combining nebivolol concentrations as described to obtain total nebivolol, individual G-UD or conjugated nebivolol concentrations is unclear because d- and l- nebivolol have different potency (pharmacological activity) and pharmacokinetics (e.g. elimination half-life). Ideally, an assay that can measure glucuronides of each enantiomer should have been developed because the enantiomers and their glucuronides are expected to have different pharmacological activities. Determination of G-UD concentration is relevant for assessing enterohepatic recirculation (EHR), as glucuronides are often involved in this pathway. However changes due to EHR are likely to be evident in the individual nebivolol enantiomers. Based on the preceding information, the drug-drug interaction reviews will primarily focus on the pharmacokinetics of the individual (non-conjugated) enantiomers, rather than total nebivolol or estimated G-UD value as the reliability of these estimates are unclear.

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4.2.18 A Phase I Open-Label Multiple-Dose Study Assessing the Pharmacokinetic Interaction of Hydrochlorothiazide and Nebivolol HCl in Healthy Volunteers

INVESTIGATORS/ Study Site	Thomas S. Clark, M.D., M.S.
STUDY PERIOD	July 26, 2002 - September 10, 2002

Summary of Drug-Drug Interaction Potential (Study Rationale)

	Hydrochlorothiazide	Nebivolol
Typical Use	Diuretic as antihypertensive agent; commonly given with other antihypertensives	Proposed for treatment of hypertension
Metabolites	None	Several metabolites including, glucuronides (major), hydroxy and oxidative metabolites
Elimination/Metabolic Pathway	Not metabolized and is eliminated unchanged primarily by renal pathways.	CYP2D6 substrate
CYP Inhibitory Potential	None identified	Low potential to inhibit CYP
Interaction Pathway/Mechanism	None expected with nebivolol	None clearly identified.
Highest Recommended Dose/Studied Dose	Individualized dosing; 25 mg dose commonly prescribed	10 mg QD

Objectives

To determine if coadministration of hydrochlorothiazide with nebivolol HCL alters the pharmacokinetics of nebivolol or hydrochlorothiazide.

Study Design

This was a randomized, open-label, multiple dose, one period, two parallel group study. Healthy subjects were genotyped to determine their CYP2D6 metabolizing status and were randomized into two groups, Group 1 and Group 2, consisting of eight subjects each (6 EM and 2 PM). The subjects received the following treatments:

Treatments

- TREATMENT A: Nebivolol Hydrochloride Tablets, 10mg (1 x 10mg) Nebivolol QD for Ten Days
- TREATMENT B: Hydrochlorothiazide Capsules, 25mg (2 x 12.5mg) Hydrochlorothiazide QD for Ten Days
- TREATMENT AB: Nebivolol Hydrochloride, 10 mg Tablets plus Hydrochlorothiazide Capsules, 25 mg for Ten Days

Group	Treatment Sequence			Subject	CYP2D6 Metabolic Status
1	A	AB	B	1, 4, 7, 8, 9, 10	EM
				14, 15	PM
2	B	AB	A	2, 3, 5, 6, 11, 12	EM
				13, 16	PM

Group 1 received Treatment A on Days 1-10, Treatment AB on Days 11-20 and Treatment B on Days 21-30. Group 2 received Treatment B on Days 1-10, Treatment AB on Days 11-20 and Treatment A on Days 21-30.

All doses of nebivolol and hydrochlorothiazide were given with 240mL of ambient temperature water. Treatments were given in the fasted state: subjects fasted at least 10 hours prior to dosing until 4 hours after dosing on Day 10, Day 20, and Day 30. Standard meals were provided the evening prior to dosing and at 4 and 10 hours after dosing on Day 10, Day 20 and Day 30. Subjects were instructed to drink plenty of water (at least six to eight 8 ounce glasses) after they left the facility to protect against possible dehydration induced by diuretic use.

Subject Characteristics

Thirteen (10 EM and 3 PM) healthy, non-smoking, male and female subjects between the ages of 19 and 45 completed this study.

Blood Sampling

- Days 1, 8, 9, 18, 19, 28 and 29: predose blood samples were collected
- Day 10: blood samples were collected at 0, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 14 and 24 hours after dosing.
- Day 20: blood samples were collected at 0, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 14 and 24 hours after dosing.
- Day 30: blood samples were collected at 0, 0.25, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 14, 24, 48, 72 and 96 hours after dosing.

Analytical Methods

Nebivolol Assay

HPLC with tandem mass spectrometry detection was used to determine nebivolol concentrations in plasma. The assay performance was acceptable as shown in Table 101.

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Table 101: Assay performance Characteristics for Nebivolol

Parameter Measure		Reviewer Comment
	Assay for Extensive Metabolizers (Curve III)	
Linearity	linear from 0.04ng/mL to 3.0ng/mL	
Precision (CV %) Within day	d-nebivolol $\leq 4.4\%$	l-nebivolol $\leq 8.2\%$
Accuracy Within day	d-nebivolol between -4.3% and 3.4%	l-nebivolol between -3.6% and 3.9%
LLOQ	0.04ng/mL	
Specificity	Chromatograms provided demonstrated assay specificity	
	Assay for Poor Metabolizers (Curve II)	
Linearity	linear from 0.2ng/mL to 15ng/mL	
Between day Precision (CV %)	d-nebivolol $\leq 4.5\%$	l-nebivolol $\leq 6.4\%$
Relative bias (between day accuracy)	d-nebivolol between -5.0% and 2.6%	l-nebivolol between 4.8% and 2.5%
LLOQ	0.2 ng/mL	
Specificity	Chromatograms provided demonstrated assay specificity	

Hydrochlorothiazide Assay

HPLC with tandem mass spectrometry detection was used to determine hydrochlorothiazide concentrations in plasma. The assay performance was acceptable as shown in Table 102.

Table 102: Assay performance Characteristics for hydrochlorothiazide

Parameter	Measure	Reviewer Comment
Range	Linear from 5 – 500 ng/mL	Satisfactory
Precision (CV %) Within day	$< 6.9\%$	Satisfactory
Accuracy Within day	varied within - 1.3% and 1.5% of the nominal concentrations.	Satisfactory
LLOQ	5 ng/mL	Satisfactory
Specificity	Chromatograms were provided demonstrating assay specificity	Satisfactory

Pharmacokinetics

Steady-state pharmacokinetic parameters for *d*- nebivolol and *l*- nebivolol and hydrochlorothiazide were calculated using noncompartmental techniques. The following PK measures were estimated: CPEAK, TPEAK, AUCTAU, CMIN, TMIN, CTROUGH, CSS, KEL, HALF, CL/ F and Vd/ F.

Statistics

Drug-drug interactions were evaluated by standard pharmaco-statistical methods. The test treatment was nebivolol + hydrochlorothiazide and the reference treatments were hydrochlorothiazide alone and nebivolol alone.

RESULTS AND DISCUSSION

Subject Disposition

Sixteen subjects were entered into the study, and thirteen subjects completed this study. Subject 1 and Subject 16 dropped out of the study due to personal reasons. Subject 8 was discontinued from the study due to pre-dose laboratory values that did not meet study requirements.

Pharmacokinetics

General

Data are presented for fourteen subjects (11 EM and 3 PM), except where indicated. The applicant combined PM and EM data for analyses because two subjects who were classified as either PM or EM exhibited a metabolic profile opposite to that predicted by the genotyping procedure. According to the applicant, the inconsistent plasma profiles apparently due to genotype misspecification, result in intrasubject variation that is inflated beyond that normally expected for either group.

Reviewer Comment on Use of Pooled Data

Ideally, the analyses should have been conducted for the EM groups and PM groups with and without the subjects with genotype misspecification, rather than pooling the data. It is noted that only 3 PMs were enrolled in the study, thus it may not have been practical to conduct separate analyses for the PMs. The use of pooled data is acceptable because it may be reflective of what may occur in clinical practice, where PMs and EMs will receive the drug.

d-nebivolol

The mean concentration versus time profile for *d*-nebivolol is illustrated graphically in Figure 68.

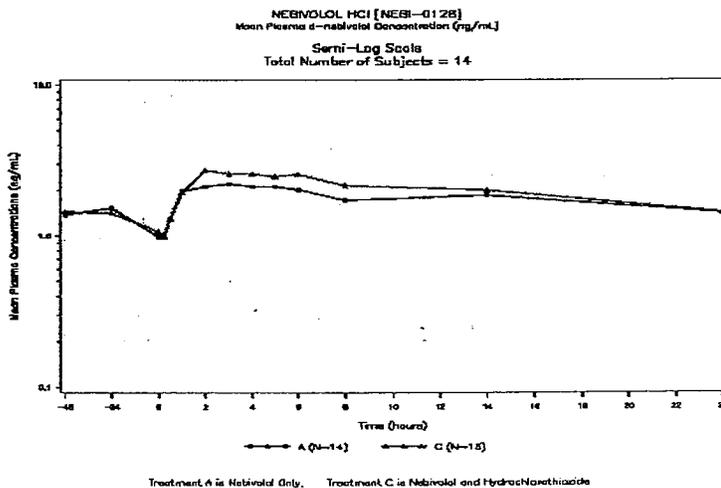


Figure 68: Plasma concentration-time profile of *d*-nebivolol +/- hydrochlorothiazide

Pharmacokinetic data for d- nebivolol are presented in Table 103.

Table 103: Mean (%CV) d-Nebivolol Pharmacokinetic Parameters in Fourteen Healthy Male and Female Subjects Following a Daily Oral Dosing of 10mg Nebivolol HCL for Ten Days Alone or Concomitantly with Daily Oral Dosing of 25mg Hydrochlorothiazide

Parameter	Treatment A Nebivolol	Treatment C Nebivolol + HCTZ	LSMEANS* Ratio (C/A)	90% Confidence Interval
CPEAK (ng/mL)	2.566 (126.8)	3.225 (134.1)	1.08	88% - 133%
CSS (ng/mL)	1.792 (166.8)	2.020 (161.2)	1.04	83% - 130%
AUCTAU (ng x hr/mL)	43.00 (166.8)	48.49 (161.2)	1.04	83% - 130%
CTROUGH (ng/mL)	0.987 (186.3)	1.072 (173.6)		
CMIN (ng/mL)	0.949 (194.0)	0.970 (187.0)		
KEL (hr ⁻¹)	0.052 (31.96)	0.064 (40.58)		
HALF (hr)	14.49 (29.48)	12.80 (42.86)		
CL/F (L/hr)	908.9 (96.39)	828.6 (88.87)		
Vd/F (L)	22016 (107.5)	15006 (105.9)		
TPEAK (hr)	3.286 (107.7)	2.615 (59.52)		

Concomitant administration of hydrochlorothiazide with nebivolol does not produce statistically significant changes in d- nebivolol pharmacokinetic parameters based on ANOVA analysis, except for KEL.

l-nebivolol

The mean concentration versus time profile for l-nebivolol is illustrated graphically in Figure 69.

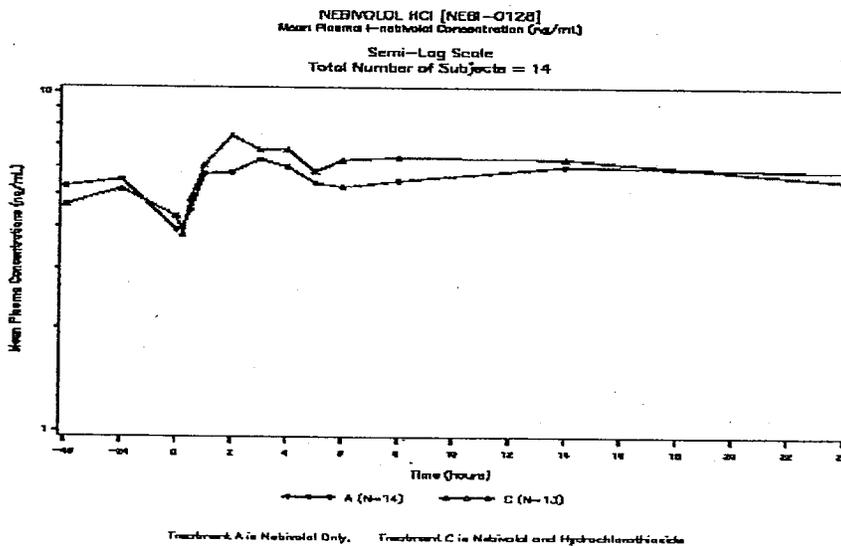


Figure 69: Plasma concentration-time profile of l-nebivolol +/- hydrochlorothiazide

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Pharmacokinetic data for l- neбиволol are presented in Table 104.

Table 104: Mean (%CV) l-Nebivolol Pharmacokinetic Parameters in Fourteen Healthy Male and Female Subjects Following a Daily Oral Dosing of 10mg Nebivolol HCL for Ten Days Alone or Concomitantly with Daily Oral Dosing of 25mg Hydrochlorothiazide

PK Measure	Treatment A Nebivolol	Treatment C Nebivolol + HCTZ	LSMEANS* Ratio (C/A)	90% Confidence Interval
CPEAK (ng/mL)	7.371 (161.1)	8.241 (154.3)	1.02	82% - 127%
CSS (ng/mL)	5.771 (192.8)	6.177 (179.8)	1.05	85% - 129%
AUCTAU (ng x hr/mL)	138.5 (192.8)	148.3 (179.8)	1.05	85% - 129%
CTROUGH (ng/mL)	3.917 (201.1)	4.301 (184.5)		
CMIN (ng/mL)	3.828 (204.2)	3.775 (189.6)		
KEL (hr ⁻¹)	0.036 (35.78)	0.044 (42.90)		
HALF (hr)	21.83 (42.90)	19.54 (55.78)		
CL/F (L/hr)	440.3 (80.61)	386.0 (82.97)		
Vd/F(L)	20399 (90.99)	12853 (107.0)		
TPEAK (hr)	4.357 (155.3)	3.000 (113.9)		
TMIN (hr)	1.804 (354.3)	0.154 (105.7)		

Concomitant administration of hydrochlorothiazide with neбиволol does not produce statistically significant changes in l- neбиволol pharmacokinetic parameters based on ANOVA analysis.

The mean concentration versus time profile for hydrochlorothiazide is illustrated graphically in Figure 70.

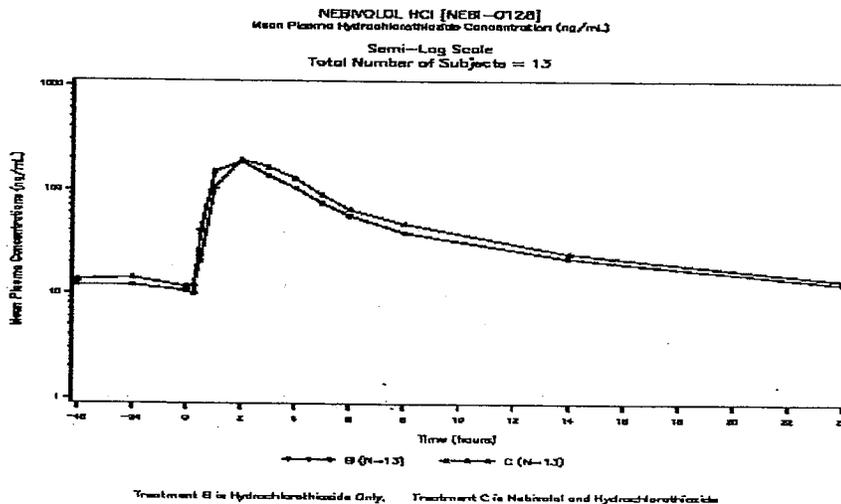


Figure 70: Plasma concentration-time profile of hydrochlorothiazide +/- l-neбиволol

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Pharmacokinetic data for hydrochlorothiazide are presented in Table 105.

Table 105: Mean (%CV) Hydrochlorothiazide Pharmacokinetic Parameters in Fourteen Healthy Male and Female Extensive Metabolizers Following a Daily Oral Dosing of 25mg Hydrochlorothiazide for Ten Days Alone or Concomitantly with Daily Oral Dosing of 10mg Nebivolol HCL

Parameter	Treatment B Hydrochlorothiazide	Treatment C Nebivolol + HCTZ	LSMEANS Ratio (C/B)	90% Confidence Interval
CPEAK (ng/mL)	196.7 (38.17)	202.6 (28.77)	1.06	96% - 116%
CSS (ng/mL)	46.21 (25.16)	50.62 (27.63)	1.09	102% - 115%
AUCTAU (ng x hr/mL)	1109 (25.16)	1215 (27.63)	1.09	102% - 115%
CTROUGH (ng/mL)	10.61 (31.64)	11.75 (47.49)		
CMIN (ng/mL)	9.089 (43.85)	11.20 (44.93)		
KEL (hr ⁻¹)	0.073 (10.61)	0.078 (13.47)		
HALF (hr)	9.588 (10.21)	9.005 (14.88)		
CL/F (L/hr)	23.64 (20.89)	21.93 (25.22)		
Vd/F (L)	323.3 (27.50)	286.0 (30.10)		
TPEAK (hr)	1.769 (24.79)	2.000 (28.87)		
TMIN (hr)	3.827 (234.0)	1.942 (341.3)		

Based on the GMR and 90 % CI, nebivolol does not alter the PK of hydrochlorothiazide.

CONCLUSIONS

Concomitant administration of 10 mg nebivolol HCL and 25 mg hydrochlorothiazide produces no pharmacokinetic changes in the rate and extent of nebivolol and hydrochlorothiazide absorption.

Labeling Recommendations

The combination of nebivolol and hydrochlorothiazide may be safely prescribed to hypertensive patients without dosage adjustment. The applicant's proposed labeling is acceptable: no pharmacokinetic interaction is observed between nebivolol and hydrochlorothiazide.

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4.2.19 A Phase I Open- Label Study Comparing the Interaction of Nebivolol HCl on the Pharmacokinetics of Digoxin in Healthy Volunteers

INVESTIGATOR(S)	Thomas S. Clark, M.D., M.S.
AND STUDY SITE	
STUDY PERIOD	October 7, 2002 - March 13, 2003

Objective

To determine the interaction of nebivolol HCL on the steady-state pharmacokinetics of digoxin.

Study Design

This was a one-period, one sequence, two treatment pharmacokinetic study. Subjects were genotyped to determine their CYP2D6 metabolizing status. All subjects received the following treatments:

Day 1: 0.25mg (1 x 0.25mg) digoxin BID

Day 2-17: 0.25mg (1 x 0.25mg) digoxin QD

Day 8-17: 10mg (1 x 10mg) nebivolol QD

All doses of nebivolol and digoxin were given with 240 mL of ambient temperature water.

Subjects fasted at least 10 hours prior to dosing until 4 hours after dosing on Day 7 and Day 17.

Subjects received additional standardized meals and snacks throughout the study and water intake was controlled.

Subject Demographics

Sixteen subjects enrolled in this study and thirteen subjects completed the study. Subject 6, Subject 15 and Subject 16 were discontinued from the study due to low pulse rates. Selected demographic characteristics are listed below.

Age Range: 20 – 53 years

Sex: 14 male, 2 female

Race: 13 white, 2 Black, 1 Oriental

Weight: 136 – 206 lb.

Blood Sampling

- Days 1, 5 and 6: blood sample was taken prior to dosing
- Day 7: serial blood samples were collected at 0, 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, 8.0, 12 and 24 hours after dosing.
- Days 3, 7, 9, 12 and 14: samples were taken at 8 hours post-dose
- Days 15 and 16: samples were collected prior to dosing
- Day 17: samples were collected at 0, 0.5, 1.0, 1.5, 2.0, 3.0, 5.0, 8.0, 12, 24, 48, 72 and 96 hours after dosing.

FORMULATIONS

- Nebivolol HCL Tablets, 10mg Mylan Pharmaceuticals Inc. Lot # R1H1182
- Digitek® (digoxin), 0.25mg Bertek Pharmaceuticals Inc. Lot # 2096A1

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Assay**d- and l-nebivolol**

Plasma samples were analyzed for d- and l-nebivolol concentrations by HPLC with tandem mass spectrometric detection. The assay performed acceptably as summarized in Table 106.

Table 106: L and D-nebivolol Assay Characteristics

Parameter	Measure	Reviewer Comment
	Assay for Extensive metabolizers (Curve III)	
Linearity	0.04ng/mL to 3.0ng/mL	Satisfactory
Precision	d-nebivolol was 4.2% or less	I-nebivolol was 4.8% or less
		Satisfactory
Accuracy	d-nebivolol -7.8% and 3.9%	d-nebivolol -7.2% and 3.3%
		Satisfactory
LLOQ	0.04ng/mL	Satisfactory
Specificity	provided that demonstrate assay is specific	Satisfactory
	Assay for Poor Metabolizers (Curve II)	
Linearity	linear from 0.2ng/mL to 15ng/mL	Satisfactory
CV Between day	d-nebivolol was 6.5% or less of nominal concentration	I-nebivolol was 5.7% or less of nominal concentration
Precision		
		Satisfactory
Relative Bias	d-nebivolol -4.8% and 6.8% of nominal concentration	I-nebivolol -5.1% and 6.2% of nominal concentration
Between day		
Accuracy		Satisfactory
LLOQ	0.2ng/mL	Satisfactory
Specificity	provided that demonstrate assay is specific	Satisfactory

Digoxin Assay

Plasma samples were analyzed for digoxin concentrations by an Immunoassay kit _____ with methodology specific to the _____ automated clinical chemistry analyzer. Assay performance was acceptable as shown in Table 107.

Table 107: Digoxin Assay Characteristics

Parameter	Measure	Reviewer Comment
	Assay for Extensive metabolizers (Curve III)	
Range	0.325 – 6.0 ng/mL	Satisfactory
Precision	4.7% or less.	Satisfactory
Accuracy	17.3, 12, 5- all values higher than nominal concentrations (check to see if typical for this assay)	Satisfactory
		Satisfactory
LLOQ	0.325ng/mL	Satisfactory
ULOQ	6.0 ng/mL	Satisfactory
Specificity	Could not be conclusively determined	one

Pharmacokinetics Analyses

Steady-state pharmacokinetic parameters for d-nebivolol, l-nebivolol and digoxin were calculated using noncompartmental techniques. Pharmacokinetic measures determined included: CPEAK_{ss}, TPEAK_{ss} on Day 7 and Day 17; AUCTAU_(24 or 96 hr) on Day 7 or Day 17; CMIN and TMIN on Day 7 or Day 17; CTROUGH (predose concentrations) on Day 7 or Day 17; CSS_{avg}; KEL; and THALF.

Statistical Analyses

Standard pharmacokinetic-statistical tests were used to evaluate drug interactions.

Results and Discussion

Pharmacokinetic Analyses

General

Subject 6 and Subject 15 were discontinued prior to Day 2 digoxin dosing and, Subject 16 was discontinued from the study prior to Day 11 nebivolol + digoxin dosing. Therefore for the digoxin analysis, data are presented for fourteen subjects for Treatment A and thirteen subjects for Treatment B.

Digoxin PK

The plasma concentration-time profile for digoxin is depicted in Figure 71.

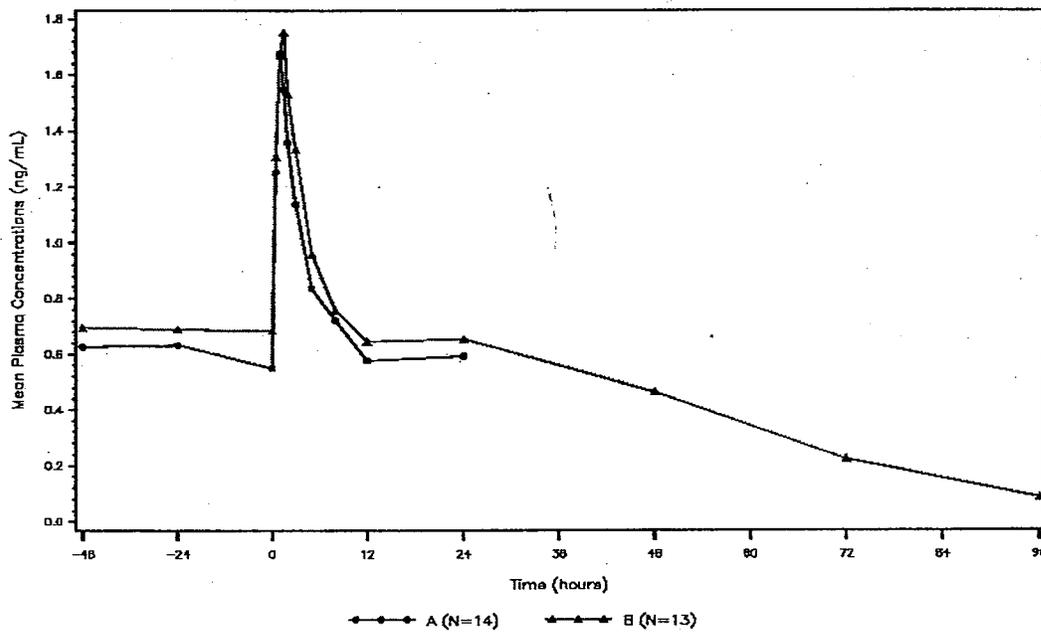


Figure 71: Mean Plasma digoxin concentration-time profile in absence (n = 14) and presence (n = 13)

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Digoxin PK measures are summarized in Table 108.

Table 108: Mean (%CV) Digoxin Pharmacokinetic Parameters in Fourteen Healthy Male and Female Subjects Following a Daily Oral Dosing of 0.25 mg Digoxin Alone or Concomitantly with Daily Oral Dosing of 10mg Nebivolol HCL

Parameter	Treatment A Digoxin (n = 14)	Treatment B Digoxin + Nebivolol (n = 13)	LSMEANS* Ratio (B/A)	90% Confidence Interval** (%)
CPEAK (ng/mL)	1.758 (22.88)	1.911 (23.74)	1.07	94 - 121
CSS (ng/mL)	0.746 (21.80)	0.826 (21.05)	1.08	103 - 114
AUCTAU (ng x hr/mL)	17.89 (21.80)	19.83 (21.05)	1.08	103 - 114
CTROUGH (ng/mL)	0.551 (36.29)	0.684 (27.32)		
CMIN (ng/mL)	0.519 (35.99)	0.612 (24.61)		
CL/F (L/hr)	14.62 (22.53)	13.13 (21.21)		
TPEAK (hr)	1.071 (40.34)	1.192 (36.47)		
TMIN (hr)	8.571 (101.7)	10.15 (81.40)		

Based on the ratio of geometric means and the 90 % confidence intervals, nebivolol does not affect digoxin PK.

Nebivolol PK

For the nebivolol analysis, data are presented for thirteen subjects. The metabolic status of Subject 1 was unclear: the plasma concentration-time profile for Subject 1 was more consistent with PM subjects (reference Study NEBI-0270*) despite having a CYP2D6 metabolic status as an EM. The applicant concluded that the metabolic status of Subject 1 was a PM thus, there were 12 EM and 1 PM. Subsequent analysis were conducted with and without the presumption that Subject 1 was a PM.

* Study NEBI-0270: A phase 1 open label multiple dose study assessing the pharmacokinetics of nebivolol HCL in healthy volunteers.

Reviewer's Comment

The applicant's designation of Subject 1 as a PM appears reasonable, however, it calls into question the specificity or selectivity of the metabolizing test: there appears to be a potential for obtaining false positives with respect to an individual's metabolic status.

The mean concentration versus time profiles for d-nebivolol and l-nebivolol in EM subjects (without Subject 1) are illustrated in Figure 72 and Figure 73, respectively. Table 109 and Table 110 summarize nebivolol PK for EM subjects, with and without Subject 1.

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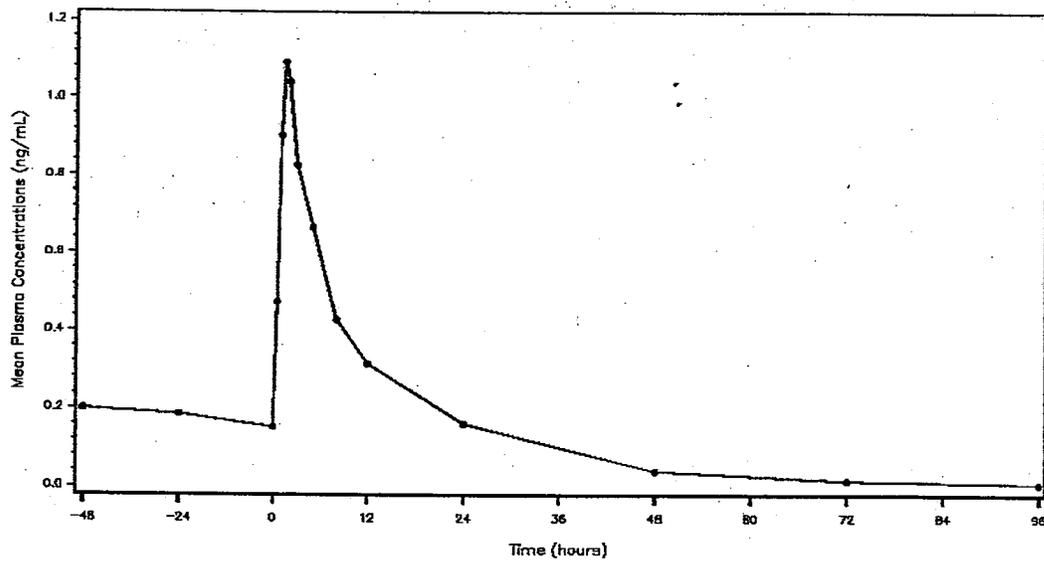


Figure 72: Mean Plasma d-nebivolol concentration-time profile in presence of digoxin (n = 11)

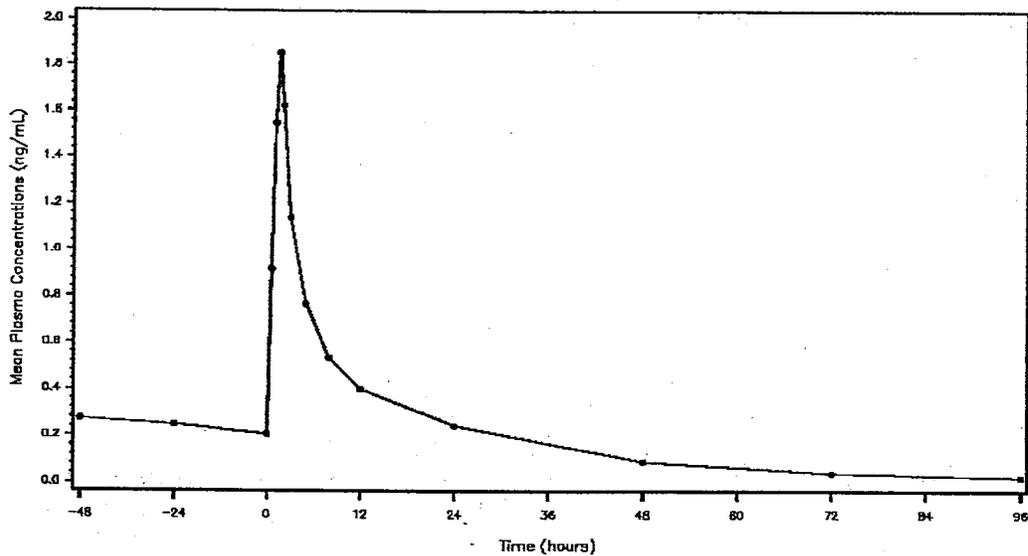


Figure 73: Mean Plasma l-nebivolol concentration-time profile in presence of digoxin (n = 11)

Concomitant administration of nebivolol with digoxin does not produce statistically significant changes in digoxin pharmacokinetic parameters.

Study Design Note

According to the applicant, the effect of steady-state digoxin on the pharmacokinetics (PK) of nebivolol was not studied due to safety concerns. Consequently, historical nebivolol data were used for comparison of nebivolol PK.

Table 109: Mean (%CV) *d*-, and *l*-Nebivolol Pharmacokinetic Parameters in Twelve Healthy Male and Female Extensive Metabolizers Following a Daily Oral Dosing of 0.25mg Digoxin Alone or Concomitantly with Daily Oral Dosing of 10mg Nebivolol HCL

Parameter	<i>d</i> -nebivolol	<i>l</i> -nebivolol
CPEAK (ng/mL)	2.552 (178.9)	5.142 (203.9)
CSS (ng/mL)	1.503 (253.1)	3.325 (287.8)
CTROUGH (ng/mL)	1.005 (295.1)	2.569 (318.8)
CMIN (ng/mL)	0.864 (290.7)	2.112 (313.1)
AUCTAU (ng x hr/mL)	36.07 (253.1)	79.80 (287.8)
KEL (hr ⁻¹)	0.079 (47.16)	0.046 (45.12)
HALF (hr)	12.23 (87.54)	26.08 (137.9)
CL/F (L/hr)	796.6 (70.21)	447.6 (50.70)
Vd/F _T (L)	9001 (52.19)	9569 (57.37)
TPEAK (hr)	1.833 (62.98)	1.458 (27.19)
TMIN (hr)	10.04 (122.7)	4.042 (230.7)

Table 110: Mean (%CV) *d*-, and *l*-Nebivolol Pharmacokinetic Parameters in Eleven Healthy Male and Female Extensive Metabolizers Following Daily Oral Dosing of 10mg Nebivolol Concomitantly with 0.25mg Digoxin for 10 Days (Subject 1 Excluded)

Parameter	<i>d</i> -nebivolol	<i>l</i> -nebivolol
CPEAK (ng/mL)	1.254 (66.17)	2.124 (39.45)
CSS (ng/mL)	0.413 (120.2)	0.566 (85.75)
CTROUGH (ng/mL)	0.151 (157.7)	0.206 (123.0)
CMIN (ng/mL)	0.142 (171.8)	0.204 (124.0)
AUCTAU (ng x hr/mL)	9.916 (120.2)	13.57 (85.75)
KEL (hr ⁻¹)	0.084 (38.95)	0.050 (34.25)
HALF (hr)	9.311 (39.62)	15.91 (48.56)
CL/F (L/hr)	867.6 (60.72)	487.7 (38.58)
Vd/F _T (L)	9729 (42.71)	10327 (48.96)
TPEAK (hr)	1.545 (39.50)	1.455 (28.57)
TMIN (hr)	10.91 (114.9)	4.364 (222.5)

The exclusion of data from Subject 1 had a significant impact on the PK findings, particularly the exposure: ~ doubles CPEAK and quadruples AUC.

Applicant's Safety Conclusions

All adverse events were listed as mild in severity, except for one instance of a moderately stiff neck. There were no serious or life threatening adverse events reported for this study.

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Summary and Conclusions

- Based on a cross-study comparison (Study 0174 vs. 0270), the steady-state pharmacokinetics for d-nebivolol and l-nebivolol in EM are comparable to data previously obtained from a multiple-dose pharmacokinetic study performed by Mylan (NEBI-0270).
- Administration of nebivolol HCL resulted in no clinically significant changes in the pharmacokinetics of digoxin.

Labeling Recommendations

The applicant's labeling language is acceptable. The language simply reflects the study design and findings indicating that nebivolol does not alter digoxin pharmacokinetics.

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4.2.20 A Phase I Open- Label Study Comparing the Interaction of Steady- state Nebivolol HCl on the Pharmacokinetic and Pharmacodynamics of Warfarin Sodium in Healthy Volunteers

INVESTIGATORS	Thomas S. Clark, M.D., M.S.
STUDY PERIOD	September 27, 2002 - December 6, 2002

Summary of Drug-Drug Interaction Potential (Study Rationale)

	Warfarin Sodium	Nebivolol
Typical Use	Anticoagulant; reduce the risk of adverse events (e.g., death, recurrent myocardial infarction) associated with myocardial infarction	Proposed for treatment of hypertension
Miscellaneous Information	Warfarin is a racemic mixture of R- and S-enantiomers. S-enantiomer exhibits 2-5 times more anticoagulant activity than the R- enantiomer, but generally has more rapid clearance.	
Metabolites (activity)	Inactive hydroxylated and warfarin alcohols	Several metabolites including, glucuronides (major), hydroxy and oxidative metabolites
Metabolic Pathway	CYP-450 enzymes involved include 2C9, 2C19, 2C8, 2C18, 1A2, and 3A4. Stereoselectively metabolism occurs via hydroxylation and reductases	CYP2D6 substrate
CYP Inhibitory Potential	None reported	Low potential to inhibit CYP
Interaction Pathway/Mechanism	None expected with nebivolol	None clearly identified.
Highest Recommended Dose/Studied Dose	Individualized therapy. Most patients receive doses of 2 to 10 mg daily	Individualized, initial is 5 mg QD but expect maintenance of 10 mg QD

Objective

To determine the interaction of steady-state nebivolol HCL on the single dose pharmacokinetics and pharmacodynamics of warfarin.

Study Design

This was an open-label, one-period, one-sequence, two-treatment study. Sixteen, non-smoking, adult, male and female volunteers between the ages of 19 and 50 were accepted into the clinical phase of this study. Subjects were genotyped to determine their CYP2D6 metabolizing status.

All subjects received the following treatments:

Day 1: Dosing: 10mg (1 x 10mg) warfarin QD
 Day 8-22: Dosing: 10mg (1 x 10mg) Nebivolol QD
 Day 17: Dosing: 10mg (1 x 10mg) Nebivolol and 10mg (1 x 10mg) warfarin QD

Subjects

Race: 15 white, 1 Hispanic
 Sex: 7 females and 9 males
 Age: 20 – 50 years
 Weight: 169 – 215 lbs.

Formulations

- Nebivolol HCL Tablets, 10 mg, Mylan Pharmaceuticals Inc. Lot # R1H1182
- Coumadin® (warfarin sodium), 10 mg, Dupont Pharma, Lot # EPL453A

Blood Sampling for PK

- Day 1: blood samples were collected predose (within 30 minutes prior to dosing) and 0.5, 1.0, 2.0, 4.0, 8.0, 12, 24, 36, 48, 72, 96, 120 and 144 hours.
- Day 15, 16 and 17: blood samples were collected prior to dosing and 2 hours post dosing
- Day 17: blood samples were collected predose (within 30 minutes prior to dosing) and 0.5, 1.0, 2.0, 4.0, 8.0, 12, 24, 36, 48, 72, 96, 120 and 144 hours post dose.

Blood Sampling for Plasma Protein Binding (PPB)

Day 1 and Day 17: blood samples were collected at the 2 hr sampling time point for determination of the PPB of warfarin.

Prothrombin time and/or INR measurements

Day 1 and Day 17: blood samples were collected at 0, 12, 24, 36, 48, 72, 96, 120 and 144 hours.

Analytical Methods*d-* and *l*-neбиволol

A high performance liquid chromatography with tandem mass spectrometric detection was used to determine *d*-neбиволol and *l*-neбиволol concentrations in human plasma (heparin). The assay performance was acceptable as shown in Table 111.

Table 111: Assay Characteristics for *d*- and *l*-Nebivolol

Parameter	Measure		Reviewer Comment
	Assay for Extensive Metabolizers (Curve III)		
Linearity	linear from 0.04ng/mL to 3.0ng/mL		Satisfactory
CV (%) Between day Precision	<i>d</i> -neбиволol ≤ 4.4 %	<i>l</i> -neбиволol ≤ 5.5	Satisfactory
Relative Bias Between day Accuracy	<i>d</i> -neбиволol within -7.7% and 3.1%	<i>l</i> -neбиволol within -2.2% and 3.9%	Satisfactory
LLOQ	0.04ng/mL		Satisfactory
Specificity	Chromatograms provided that demonstrate assay specificity		Satisfactory
	Assay for Poor Metabolizers (Curve II)		
Linearity	linear from 0.2ng/mL to 15ng/mL		Satisfactory
CV (%) Between day Precision	<i>d</i> -neбиволol ≤ 5.9 %	<i>l</i> -neбиволol ≤ 5.8	Satisfactory
Relative Bias Between day Accuracy	<i>d</i> -neбиволol within -3.9% and 4.9%	<i>l</i> -neбиволol within -2.3% and 3.4%	Satisfactory
LLOQ	0.2ng/mL		Satisfactory
Specificity	Chromatograms provided that demonstrate assay specificity		Satisfactory

R- and S-warfarin

A stereoselective, high performance liquid chromatography with tandem mass spectrometric detection method was used to determine R- and S-warfarin concentrations. The assay performance was acceptable as shown in Table 112.

Table 112: Assay Characteristics for R- and S-warfarin

Parameter	Measure	Reviewer Comment
Linear range	linear from 1.00 ng/mL to 100.0 ng/mL	Satisfactory
Precision (CV %) Within day	R-warfarin < 22.4%	S-warfarin ≤ 22.2% % Satisfactory
Accuracy Within day	within -6.9% and 4.0%	varied within -8.3% and 1.2% Satisfactory
LLOQ	1.00 ng/mL	Satisfactory
Specificity	Chromatograms provided that demonstrate assay specificity	Satisfactory

Pharmacokinetics

Single-dose pharmacokinetic parameters for R-warfarin and S-warfarin were calculated using noncompartmental techniques. PK measures calculated were: CPEAK, TPEAK, KEL, AUCL, AUCI, THALF, CL/F (Dose/AUCI), and Vd/F.

Pharmacodynamics

The degree of anticoagulation produced by warfarin administration was determined by International Normalized Ratio (INR). Pharmacodynamic parameters for prothrombin time and INR were calculated using noncompartmental techniques. The maximum concentration (PTPEAK and INRPEAK, for prothrombin time and INR, respectively) and the time at which it occurred relative to the administered dose (TPEAK) were determined from the observed prothrombin time-time or INR-time profiles over the sampling time interval. Area under the prothrombin time-time or INR-time curves (PTAUCT or INRAUCT) was the sum of the linear trapezoidal estimation of the areas from the time of dosing to the time of the last recorded measurement (144 hours).

Protein Binding

The degree of plasma protein binding to warfarin was determined by standard ultrafiltration techniques using radiolabeled warfarin.

Statistics

- For Nebivolol Concentrations

d-neбиволol and *l*-neбиволol concentrations were compared at the following time points: 46 hours prior to and 22 hours prior to dosing on Day 17 (Treatment C) and 2 hours post dosing on Day 17 (Treatment B).

- For Warfarin Pharmacokinetics

Standard pharmaco-statistical analyses were used to determine if warfarin underwent a drug-drug interaction when coadministered with neбиволol. The test treatment was warfarin + neбиволol and the test treatment was warfarin alone.

- For Warfarin Pharmacodynamics

The following PD measures were analyzed in a manner similar to that for evaluating PK drug interactions: PTAUCT, PTPEAK and TPEAK (for prothrombin time) and INRAUCT, INRPEAK and TPEAK (for INR), LNPTAUCT, LNPTPEAK, LNINRAUCT, and LNINRPEAK.

Results and Discussion

Sixteen subjects were entered in this study and fourteen subjects completed the study. Subject 12 and Subject 13 were discontinued from the study due to abnormal laboratory values prior to Day 17 dosing.

Pharmacokinetic Analyses

General Information

Plasma concentration data are presented for all sixteen subjects and with Subjects 12, 13, 15 and 16 deleted for *d*-neбиволol and *l*-neбиволol, R-warfarin, and for S-warfarin. Subjects 12 and 13 were discontinued from the study prior to Day 17 dosing and therefore did not receive the concomitant administration of warfarin and neбиволol. Subjects 15 and 16 do not have a complete pharmacokinetic profile for the concomitant treatment of warfarin and neбиволol due to a dosing error by the clinical site. PK data in this report exclude incomplete data collected for Subjects 12, 13, 15 and 16.

The applicant combined data from EM and PM subjects because warfarin is not metabolized by CYP2D6 and subject to genetic polymorphism. According to the applicant visual inspection of data confirms this assumption. Although this approach is reasonable, it would have been more appropriate to analyze the data separately, in case the metabolic status impacts the potential neбиволol-warfarin interaction.

d- and *l*-neбиволol

Formal PK analyses were not conducted on *d* and *l* neбиволol due to the limited blood sampling. However, based on plasma concentration data obtained two hours after dosing on Day 16 and Day 17, concomitant administration of neбиволol with warfarin does not produce statistically significant changes in *d*-neбиволol and *l*-neбиволol plasma concentrations.

Reviewer's Comment

This approach is not acceptable to form definitive conclusions regarding warfarin's effect on neбиволol PK. The Drug-Drug Interaction *Guidance for Industry* recommends that drug-drug interactions be evaluated by specific statistical exposure (AUC and C_{max}) comparisons, not based on single time-point comparisons.

R-warfarin

The mean concentration versus time profile for R-warfarin is illustrated graphically in Figure 74.

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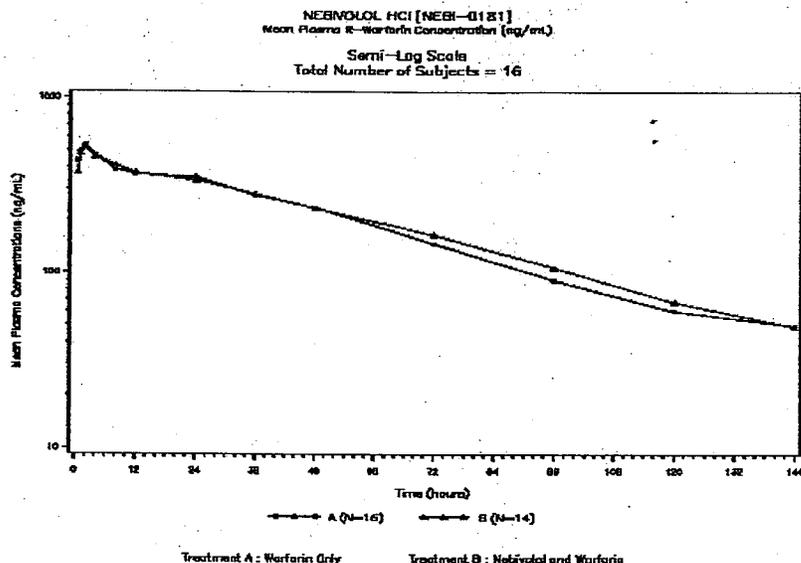


Figure 74: Mean R-warfarin plasma concentration-time profile

Pharmacokinetic data for R-warfarin are presented in Table 113.

Table 113: Mean (%CV) R-Warfarin Pharmacokinetic Parameters in Twelve Healthy Male and Female Subjects Following a Single, Oral Dose of 10 mg Coumadin® Given Alone or Concomitantly with 10mg Nebivolol HCL Under Fasting Conditions

Parameter	Arithmetic Mean Treatment A (warfarin alone)	Arithmetic Mean Treatment B (warfarin + nebivolol)	LSMEANS Ratio (B/A)	90% Confidence Interval
AUCL (ng x hr/mL)	26246 (20.33)	28030 (19.67)	1.07	103% - 111%
AUCI (ng x hr/mL)	29426 (20.62)	31407 (19.01)	1.07	103% - 111%
CPEAK (ng/mL)	555.9 (21.48)	582.8 (19.86)	1.05	95% - 116%
KEL (hr ⁻¹)	0.016 (18.05)	0.016 (26.73)	-----	-----
HALF (hr)	44.34 (16.49)	46.80 (24.12)	-----	-----
TPEAK (hr)	3.625 (178.7)	1.750 (68.38)	-----	-----
CL/F (L/hr)	0.176 (19.83)	0.164 (17.34)	-----	-----
Vd/F (L)	11.36 (27.69)	11.21 (32.11)	-----	-----

Concomitant administration of nebivolol with warfarin did not cause a change in R-warfarin exposure.

The mean concentration versus time profile for S-warfarin is illustrated graphically in Figure 75.

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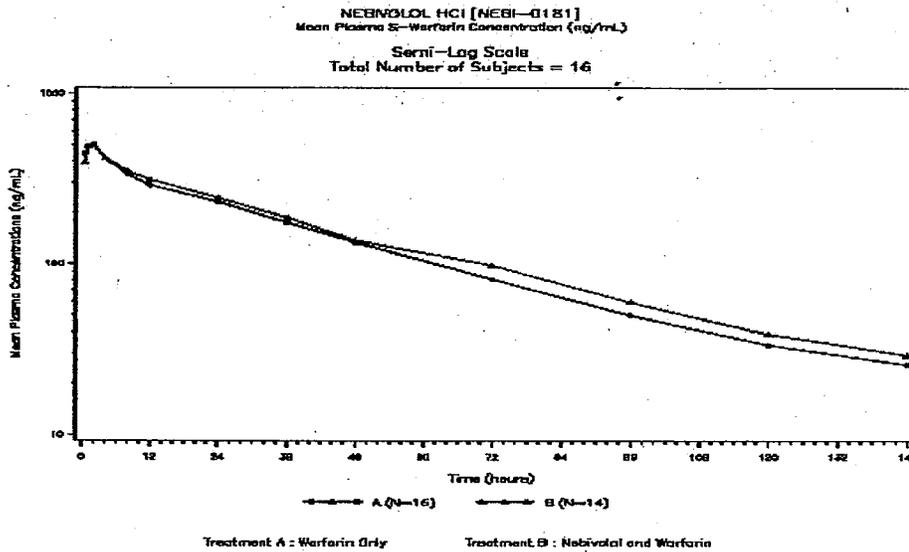


Figure 75: Mean S-warfarin plasma concentration-time profile

Pharmacokinetic data for S-warfarin are presented in Table 114.

Table 114: Mean (%CV) S-Warfarin Pharmacokinetic Parameters in Twelve Healthy Male and Female Subjects Following a Single, Oral Dose of 10mg Coumadin® Given Alone or Concomitantly with 10mg Nebivolol HCL Under Fasting Conditions

Parameter	Arithmetic Mean Treatment A (warfarin alone)	Arithmetic Mean Treatment B (warfarin + nebivolol)	LSMEANS Ratio (B/A)	90% Confidence Interval
AUCL (ng x hr/mL)	17437 (35.85)	18932 (34.48)	1.10	102% - 118%
AUCI (ng x hr/mL)	19166 (40.26)	21055 (40.75)	1.10	103% - 118%
CPEAK (ng/mL)	530.4 (22.28)	573.6 (18.17)	1.09	97% - 123%
KEL (hr ⁻¹)	0.017 (27.67)	0.017 (26.05)	-----	-----
HALF (hr)	42.56 (28.06)	43.81 (36.31)	-----	-----
TPEAK (hr)	1.582 (61.46)	1.375 (72.93)	-----	-----
CL/F (L/hr)	0.294 (32.21)	0.264 (27.60)	-----	-----
Vd/ (L)	17.70 (40.60)	16.03 (31.14)	-----	-----

Concomitant administration of nebivolol with warfarin did not cause a change in S-warfarin exposure.

Pharmacodynamic Analysis

The mean prothrombin time (PD measure) versus time profile for warfarin in the absence and presence of nebivolol is illustrated graphically in Figure 76.

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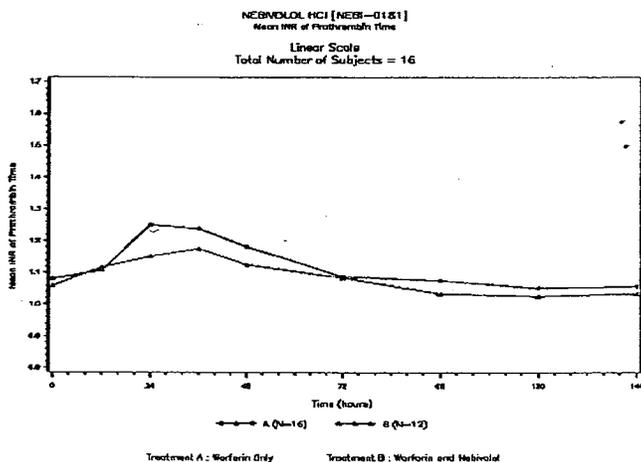


Figure 76: Warfarin prothrombin time in the absence and presence of nebivolol

Pharmacodynamic data for prothrombin time are presented in Table 115. Concomitant administration of nebivolol with warfarin decreased warfarin prothrombin time; however, the decrease in prothrombin time does not appear clinically relevant (falls within the no effect range). No changes in prothrombin time parameters. As expected, from the prothrombin time results, mean INR was not affected by nebivolol coadministration.

Table 115: Mean (%CV) Prothrombin Time Pharmacodynamic Parameters in Twelve Healthy Male and Female Subjects Following a Single, Oral Dose of 10mg Coumadin® Given Alone or Concomitantly with 10mg Nebivolol HCL Under Fasting Conditions

Parameter	Arithmetic Mean Treatment A (warfarin alone)	Arithmetic Mean Treatment B (warfarin + nebivolol)	LSMEANS Ratio (B/A)	90% Confidence Interval
PTPEAK (sec)	13.12 (11.83)	12.44 (7.938)	0.95	92% - 98%
PTAUCT (sec x hr)	1680 (7.644)	1636 (4.904)	0.97	96% - 99%
TPEAK (hr)	25.00 (24.72)	31.00 (48.00)	-----	-----

The mean INR versus time profile for warfarin in the presence and absence of nebivolol is illustrated graphically in Figure 77.

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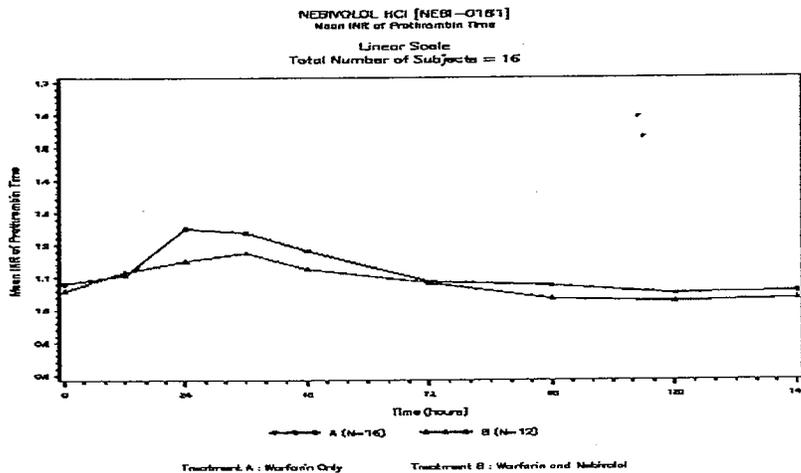


Figure 77: INR vs. time profile for warfarin in the presence and absence of nebivolol

Protein Binding Analysis

In all subjects, 99% of the warfarin present in each sample was bound to plasma proteins on Day 1 and Day 17 suggesting that nebivolol did not alter warfarin plasma protein binding.

Applicant's Summary of Safety Analysis

Laboratory, vital sign and ECG monitoring indicated no safety risk associated with oral dosing of 10mg (1 x 10mg) Coumadin® tablets concomitantly with 10mg (1 x 10mg) nebivolol HCL tablets. There were no serious or life threatening adverse events reported for this study.

Conclusions

- Administration of nebivolol HCL resulted in no clinically significant changes in the pharmacokinetics of R- or S-warfarin
- Nebivolol had no clinically significant effects on the anticoagulant activity of warfarin, as assessed by prothrombin time and INR
- Warfarin protein binding in human plasma was independent of the absence or presence of nebivolol.

Labeling Recommendations

The results of the study should be reflected in labeling. The applicant's proposed labeling is acceptable. Ideally the study should have been conducted in patients on warfarin therapy to ensure that the results will be applicable in a chronic setting. However, based on the limited interaction potential (PK and PD) between the two drugs, the single dose (warfarin) study is reasonable because it minimizes risk to study participants..

Administration of nebivolol (10 mg once daily) results in no significant changes in the pharmacokinetics of R- and S-warfarin following a single 10 mg dose of warfarin. Nebivolol has no significant effects on the anticoagulant activity of warfarin, as assessed by prothrombin time and INR profiles from 0 to 144 hours after a 10 mg single warfarin dose in 12 healthy volunteers.

4.2.21 A Phase I Open- Label Multiple- Dose Study Assessing the Pharmacokinetic Interaction Between Fluoxetine HCl and Nebivolol HCl in Healthy Volunteers

INVESTIGATORS	Thomas S. Clark, M.D
STUDY PERIOD	November 16, 2002 – December 20, 2002

Summary of Drug-Drug Interaction Potential (Study Rationale)

	Fluoxetine	Nebivolol
Metabolites	norfluoxetine and other unidentified metabolites	Several metabolites including, glucuronides (major), hydroxy and oxidative metabolites
Metabolic Pathway	CYP2D6 substrate	CYP2D6 substrate
CYP Inhibitory Potential	CYP2D6 inhibitor	Low potential to inhibit CYP
Coadministration Recommendation	if adding a CYP2D6 substrate to fluoxetine, initiate at the low end of its dosing range	Proposed
Highest Recommended Dose/Studied Dose	80 mg/day via titration and 20 mg QD initially for fluoxetine naïve patients	10 mg QD

Study Objective

To determine the effect of steady-state levels of fluoxetine on the single-dose pharmacokinetic parameters of nebivolol

Study Design

Twelve healthy non-tobacco using adult volunteers were accepted into this study.

All volunteers were genotyped for CYP2D6 metabolic status prior to entry into the study. Only EMs were enrolled because they undergo the relevant for CYP2D6 interaction; PMs metabolize mainly via glucuronidation. PM subjects also take longer to obtain steady-state with fluoxetine. Each subject received the following treatments in this study:

- Day 1: A single, oral 10mg (1 x 10mg) dose of nebivolol HCL tablets
- Days 8 through 28, a 20mg (1 x 20mg) capsule dose of fluoxetine HCL QD
- Day 28, in addition to the fluoxetine dose, a single oral dose of 10mg (1 x 10mg) nebivolol HCL tablet was administered.

Treatments were given in the fasted state and standard meals were provided throughout the study. Additionally, the protocol controlled fluid intake.

Demographics

Twelve subjects were entered into this study and ten subjects completed this study. All subjects were genotyped as CYP2D6 extensive metabolizers. Selected demographic characteristics of the enrolled subjects are:

- Race White
- Sex 11 male, 2 female
- Weight 134 – 211 lb.

Reviewer Note: Fluoxetine Dose Administered

The fluoxetine dose administered is not the highest approved dose (per Drug-Drug Interaction Guidance recommendation). However, the chosen fluoxetine dose is acceptable because the time

to titrate a healthy individual up to the 80 mg/day level may not be rationale or ethical due to the excessive exposure of unnecessary amounts of fluoxetine show an interaction.

Formulations

- Nebivolol Hydrochloride 10mg Tablets, containing 10mg of free base nebivolol; Mylan Pharmaceuticals Inc. Lot # RIH1182
- Fluoxetine HCL 20mg Capsules, Mylan Pharmaceuticals Inc. Lot #IJ4051

Blood Sampling

Day 1: blood samples were collected at pre-dose (within 10 minutes prior to dosing), 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 16, 24, 36, 48, 72, 96, 120 and 144 post dose

Day 8, 26 and 27: predose blood samples were collected

Day 28: predose and 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 16, 24, 36, 48, 72, 96, 120 and 144 hours post dose.

Analytical Methods

• *d- and l-nebivolol* Assay

A high performance liquid chromatography with tandem mass spectrometric detection was used for the analysis of *d-nebivolol* and *l-nebivolol* in human plasma (heparin). The assay performance was acceptable as summarized in Table 116.

Table 116: Assay Characteristics for d- and l-Nebivolol

Parameter	Measure	Reviewer Comment
Linearity	linear from 0.04ng/mL to 3.0ng/mL	Satisfactory
CV : Between day Precision	d-nebivolol \leq 4.9 % l-nebivolol < 7.8 %	Satisfactory
Relative Bias Between day Accuracy	d-nebivolol between -8.3% and 5.7% l-nebivolol between -5.2% and 6.3%	Satisfactory
LLOQ	0.04ng/mL	Satisfactory
Specificity	Chromatograms provided that indicate assay specificity	Satisfactory

• Fluoxetine and Norfluoxetine Assay

HPLC with tandem mass spectrometric detection assay was used for analysis of fluoxetine and norfluoxetine in blood. The assay performance was acceptable as summarized in Table 117.

Table 117: Assay Characteristics for Fluoxetine and Norfluoxetine

Parameter	Measure	Reviewer Comment
Linearity	linear from .250ng/mL to 100ng/mL	Satisfactory
CV : Between day Precision	fluoxetine < 5.3 % norfluoxetine < %	Satisfactory
Relative Bias Between day Accuracy	fluoxetine between 7.4% and 5.3% norfluoxetine between -9.5% and 5.8%	Satisfactory
LLOQ	0.250ng/mL	Satisfactory
Specificity	Chromatograms provided that indicate assay specificity	Satisfactory

Pharmacokinetics

Single-dose pharmacokinetic parameters for *d*-nebivolol and *l*-nebivolol were calculated using noncompartmental techniques. PK measures determined were: CPEAK, TPEAK, KEL, AUCT, AUCI, THALF, CL/F, and Vd/F.

Statistics

Standard pharmacostatistical analyses were used to evaluate the drug-drug interaction. The treatment group was fluoxetine + nebivolol and the reference group was nebivolol alone.

RESULTS AND DISCUSSION

Pharmacokinetics

Statistical analyses were performed and presented for the ten subjects that completed all aspects of the study.

d-nebivolol

The mean concentration versus time profiles for *d*-nebivolol is illustrated graphically in Figure 78.

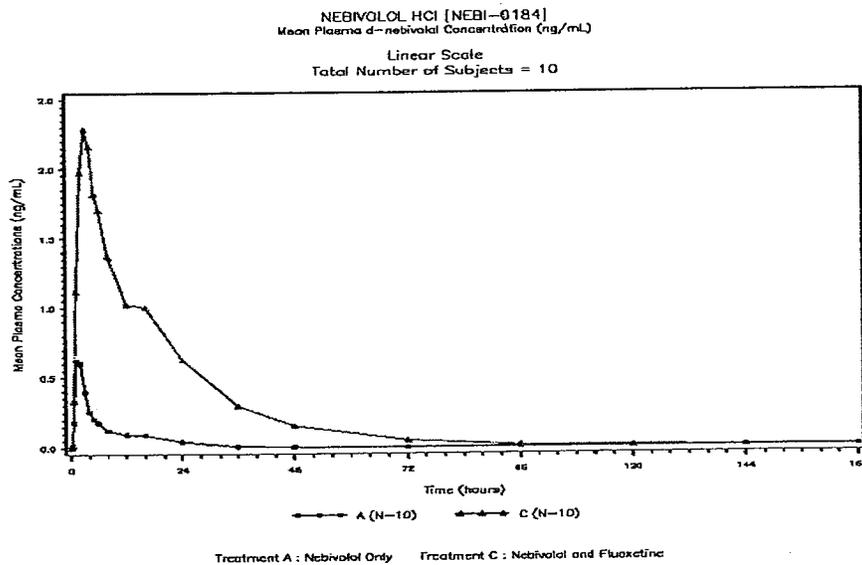


Figure 78: *d*-nebivolol plasma concentration time-profile in the absence and presence of fluoxetine

Mean (% CV) single-dose pharmacokinetic parameters of *d*-nebivolol and *l*-nebivolol in the presence and absence of fluoxetine are summarized in Table 118 and **Error! Reference source not found.**, respectively. Fluoxetine caused large increases in the exposure of *d*- and *l*-nebivolol.

Table 118: Mean (% CV) d-Nebivolol Pharmacokinetic Parameters in Ten Subjects Following a Single, Oral 10mg (1X10mg) Dose of Nebivolol Hydrochloride Tablets Under Fasting Conditions in the Presence and Absence of Fluoxetine

Parameter	Treatment A (Day 1)	Treatment C (Day 28)	Treatment C/Treatment A Least Square Mean Ratio (%)	90% Confidence Interval
AUCL (ng x hr/mL)	3.839 (53.19)	39.35 (60.51)	987	714 – 1365
AUCI (ng x hr/mL)	4.910 (43.31)	40.58 (58.82)	779	570 – 1064
CPEAK (ng/mL)	0.756 (26.21)	2.473 (34.58)	316	256 – 390
KEL (hr ⁻¹)	0.056 (26.66)	0.057 (28.35)		
HALF (hr)	13.11 (25.08)	13.21 (31.69)		
TPEAK (hr)	1.400 (49.94)	3.000 (27.22)		
CL/F (L/hr)	1166 (34.57)	159.0 (51.90)		
Vd/F (L)	21566 (37.27)	2834 (48.63)		

L-nebivolol

The mean concentration versus time profiles for L-nebivolol is illustrated graphically in Figure 79.

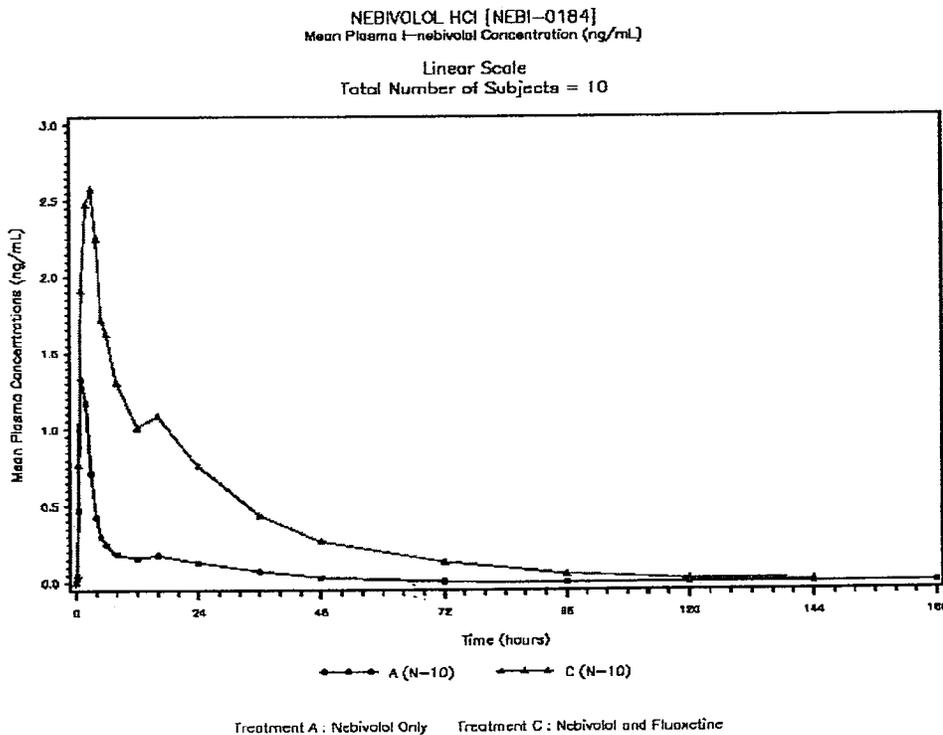


Figure 79: L-nebivolol plasma concentration time-profile in the absence and presence of fluoxetine

Table 119: Mean (%CV) l-Nebivolol Pharmacokinetic Parameters in Ten Subjects Following a Single, Oral 10mg (1X10mg) Dose of Nebivolol Hydrochloride Tablets Under Fasting Conditions in the Presence and Absence of Fluoxetine120

Parameter	Treatment A (Day 1)	Treatment C (Day 28)	Treatment C/ Treatment A Least Square Mean Ratio (%)	90% Confidence Interval
AUCL (ng x hr/mL)	8.792 (28.27)	49.91 (64.04)	507	389 – 659
AUCI (ng x hr/mL)	9.989 (25.52)	52.28 (64.93)	464	354 – 608
CPEAK (ng/mL)	1.576 (27.18)	3.079 (34.23)	189	154 – 232
KEL (hr ⁻¹)	0.044 (19.70)	0.035 (31.85)		
HALF (hr)	16.36 (21.01)	21.95 (40.37)		
TPEAK (hr)	1.300 (37.16)	2.300 (50.41)		
CL/F (L/hr)	533.0 (27.72)	127.0 (53.83)		
Vd/F (L)	12498 (34.16)	3531 (40.39)		

Reviewer's Note: Apparent Stereoselective Metabolism of Nebivolol

Upon co-administration with fluoxetine at steady-state, the pharmacokinetic parameters of nebivolol become significantly altered for all subjects. There appeared to be stereoselective metabolism as the d-nebivolol exposure was increased ~ 8-fold whereas the l-nebivolol AUC was increased ~ 5-fold. For all subjects and all nebivolol analytes, the AUCL and AUCI increased at least two-fold when fluoxetine was present.

Sponsor's Safety Summary

According to the applicant, there was no safety risk associated with a 10mg (1 x 10mg) dose of nebivolol hydrochloride tablets administered with or without fluoxetine hydrochloride.

Summary and Conclusions

Co-administration of fluoxetine decreased the apparent clearance of d- and l- nebivolol, relative to when nebivolol was administered alone, leading to increased AUCL, AUCI, and CPEAK values. The increase in exposure of d-nebivolol is approximately 8-fold for AUC and 3 fold for CPEAK and for l-nebivolol the increase is approximately 5-fold for AUC and 2-fold for CPEAK.

Labeling Recommendations

The increased nebivolol plasma concentrations observed when fluoxetine, or possibly any CYP2D6 inhibitor, is co-administered should be treated with caution by a prescribing physician. Therefore, consideration should be made to start at the lowest possible nebivolol dose and the dose adjusted based on tolerability. The applicant's proposed labeling is acceptable with minor modification. The applicant highlighted the findings for d-nebivolol, rather than l-nebivolol, thus giving the worst case scenario. The label should reflect the findings for the individual enantiomers or provide a mean value for both enantiomers.

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4.2.22 A Phase I Open-Label Multiple-Dose Study Assessing the Pharmacokinetic Interaction Between Furosemide and Nebivolol HCl in Healthy Volunteers (NEBI-0213)

INVESTIGATORS	James D. Carlson, Pharm.D.
STUDY PERIOD	December 1, 2002 to December 16, 2002

Summary of Drug-Drug Interaction Potential (Study Rationale)

	Furosemide	Nebivolol
Typical Use	diuretic for hypertension treatment, alone or with other antihypertensive agents. Also indicated for the treatment of edema associated with congestive heart failure	Proposed for treatment of hypertension
Metabolites	Furosemide glucuronide is the major metabolite in humans	Several metabolites including, glucuronides (major), hydroxy and oxidative metabolites
Metabolic Pathway	elimination occurs primarily by renal excretion	CYP2D6 substrate
CYP Inhibitory Potential	None reported	Low potential to inhibit CYP
Interaction Pathway/Mechanism	None expected with nebivolol	None clearly identified.
Highest Recommended Dose/Studied Dose	Initial dose in adult patients with hypertension not taking other diuretics is 80 mg, usually divided into 40mg twice a day (1 x 40mg, BID) (PDR 2002).	Individualized therapy, initial dose 5 mg but 10 mg QD expected to be maintenance dose

Study Objective

- Primarily to determine the effect of steady state nebivolol on the pharmacokinetics of a single coadministered dose of furosemide.
- Secondarily to assess the effects of single- dose furosemide administration on the multiple- dose pharmacokinetics of d- and l- nebivolol.

Study Design

Fifteen healthy, non- smoking, male and female subjects between the ages of 20 and 43 completed this open-label, one-period study. Subjects were genotyped to determine their CYP2D6 metabolizing status. The subjects received the following treatments:

- TREATMENT A (Days 2-11): Nebivolol Hydrochloride Tablets. Dosing: 10mg (1 x 10mg) Nebivolol QD for Ten Days
- TREATMENT B (Day 1): Furosemide Tablets. Dosing: 40mg (1 x 40mg) Furosemide QD for One Day
- TREATMENT C (Day 11):* Nebivolol Hydrochloride Tablets and Furosemide Tablets. Dosing: 10mg (1 x 10mg) Nebivolol and 40mg (1 x 40mg) Furosemide QD for One Day

Subject	CYP2D6 Metabolic Status
2, 4, 5, 6, 7, 8, 9, 10, 11, 13, 14, 15	EM
1, 3, 12	PM

All doses of nebivolol and furosemide were given with 240 mL of ambient temperature water. Treatments were given in the fasted state: subjects fasted at least 10 hours prior to dosing until at least 4 hours after dosing on Days 1, 10, and 11. Standard meals were provided on the evenings prior to dosing and at 4 and 10 hours after dosing on Days 1, 10 and 11. On Days 1 and 11, subjects were instructed to drink plenty of water in order to protect against dehydration induced by furosemide.

Blood Sampling

Day 1 blood samples (1 x 5mL) were collected prior to dosing and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 8 and 10hr post dose.

Days 8 and 9 pre- dose blood samples

Day 10: 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 12 and 24 hours.

Day 11 predose and at 0.25, 0.5, 1, 1.5, 2, 2.5, 3, 3.5, 4, 5, 6, 7, 8, 10, 12, 16, 24, 48, 72 and 96hr

Analytical Method

Nebivolol Assay

HPLC with tandem mass spectrometric detection was used to determine the concentrations of d- nebivolol and l- nebivolol in human plasma (heparin). Assay performance was acceptable as shown in Table 120.

Table 120: Assay Characteristics for d- and l-Nebivolol

Parameter	Measure	Reviewer Comment	
	Assay for Extensive Metabolizers (Curve III)		
Linearity	linear from 0.04ng/mL to 3.0ng/mL	Satisfactory	
CV : Between day Precision	d-nebivolol \leq 9.9 %	l-nebivolol \leq 13.9 %	Satisfactory
Relative Bias Between day Accuracy	d-nebivolol between -5.6 % and 5.9 %	l-nebivolol between -5.8% and 6.5 %	Satisfactory
LLOQ	0.04ng/mL	Satisfactory	
Specificity		Satisfactory	
	Assay for Poor Metabolizers (Curve II)		
Linearity	linear from 0.2ng/mL to 15ng/mL	Satisfactory	
CV : Between day Precision	d-nebivolol \leq 8.2 %	l-nebivolol \leq 9.8 %	Satisfactory
Relative Bias Between day Accuracy	d-nebivolol between -9.4 % and 10.4 %	l-nebivolol between -9.3 % and 10.0 %	Satisfactory
LLOQ	0.2ng/mL	Satisfactory	
Specificity	Chromatograms indicate that assay was specific	Satisfactory	

Furosemide Assay

HPLC with tandem mass spectrometric detection was used to determine the concentrations of furosemide in human plasma (heparin). Assay performance was acceptable as shown in Table 121.

Table 121: Assay Characteristics for furosemide

Parameter	Measure	Reviewer Comment
Linearity	linear from 0.05 μ g/ mL to 10 μ g/ mL	Satisfactory
CV : Between day Precision	\leq 11.8 %	Satisfactory
Relative Bias Between day Accuracy	between -4.4% and 6.4 %	Satisfactory
LLOQ	0.05 μ g/ mL	Satisfactory
Specificity	Chromatograms indicate that assay was specific	Satisfactory

Formulations

- Nebivolol HCL Tablets, 10mg, Mylan Pharmaceuticals Inc. Lot # R1H1182
- Furosemide Tablets, 40mg, Mylan Pharmaceuticals Inc. Lot # 1K0001

PharmacokineticsNebivolol

Steady-state pharmacokinetic (PK) parameters for d- nebivolol and l- nebivolol were calculated using noncompartmental techniques. The following PK measures were estimated: CPEAK, TPEAK, AUCTAU, CMIN, CTROUGH, CSS, KEL, CL/F, and Vd/ F.

Furosemide

The PK parameters following a single dose of furosemide (Days 1 and 11) were estimated from plasma drug concentration data using noncompartmental techniques. The following PK measures were estimated: CPEAK, TPEAK, AUCL, AUCI, KEL, HALF, CL/ F and Vd/ F.

Statistics

Drug-drug interactions were evaluated by standard pharmaco-statistical analyses. The test treatment was nebivolol + furosemide and the reference treatments were nebivolol alone and furosemide alone.

Results and DiscussionNebivolol Pharmacokinetics

Steady- state plasma concentrations were achieved by Days 8 or 9 for d- nebivolol, l- nebivolol and d, l- nebivolol.

d-Nebivolol

The mean concentration versus time profiles for d- nebivolol in EM and PMs are illustrated graphically in figure 1.

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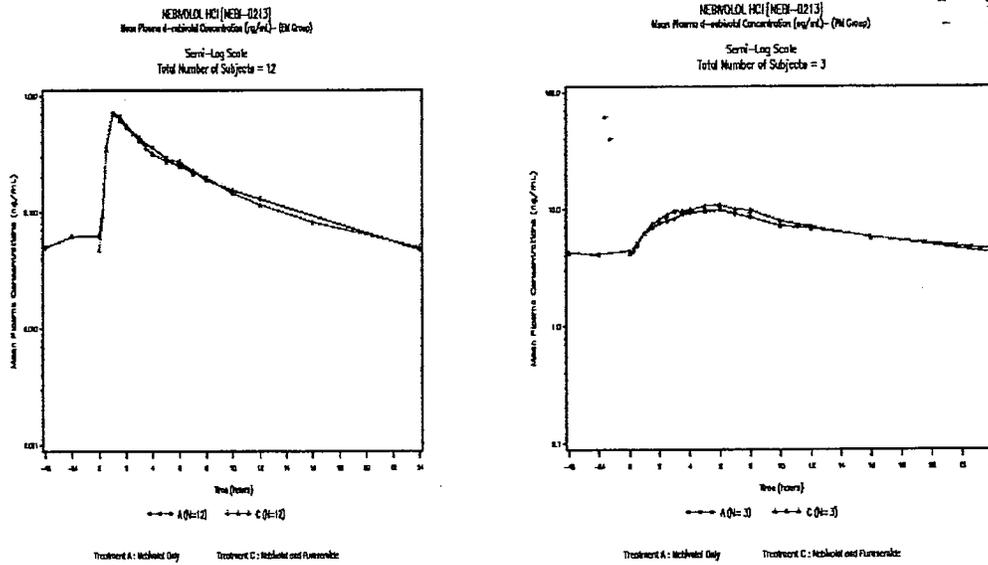


Figure 80: Plasma concentration-time profile of d-nebivolol in EMs and PMs

Based on ANOVA analysis, concomitant administration of furosemide with nebivolol did not result in a statistically significant drug-drug interaction with respect to the primary exposure measures Table 122.

Table 122: Mean (%CV) d-Nebivolol Pharmacokinetic Parameters in Twelve Healthy Male and Female Extensive Metabolizers Following a Daily Oral Dose of 10mg Nebivolol HCL for Ten Days Alone or Concomitantly with a Single Oral Dose of 40mg Furosemide

Parameter	Treatment A Nebivolol	Treatment C Nebivolol + Furosemide	LSMEAN Ratio (C/A)	90% Confidence Interval
CPEAK (ng/mL)	0.775 (32.6)	0.773 (24.9)	1.01	92% - 111%
CSS (ng/mL)	0.194 (54.0)	0.181 (44.9)	0.97	91% - 103%
AUCTAU (ng·hr/mL)	4.647 (54.0)	4.336 (44.9)	0.97	91% - 103%
CTROUGH (ng/mL)	0.063 (87.4)	0.048 (95.2)	0.82	74% - 91%
CMIN (ng/mL)	0.048 (95.4)	0.045 (98.8)	-	-
KEL (hr ⁻¹)	0.080 (26.3)	0.063 (30.8)	-	-
HALF (hr)	9.1 (21.1)	12.1 (37.6)	-	-
CL/F (L/hr)	1334 (43.5)	1329 (34.6)	-	-
Vd/F (L)	17,240 (47.3)	23,821 (64.8)	-	-
TPEAK (hr)	1.292 (50.8)	1.250 (40.0)	-	-
TMIN (hr)	16.000 (73.9)	6.000 (180.9)	-	-

There was a 21% decrease in the mean apparent KEL for d- nebivolol, when nebivolol HCL (10mg) was given with furosemide (40mg). However, the change in KEL does not appear to be clinically significant. According to the applicant, the changes seen in apparent KEL and HALF were due to the inherent variability seen in low plasma

concentrations observed for d- nebivolol in EM subjects during the drug's elimination phase. This explanation seems plausible.

PK measures for d-nebivolol in PMs are summarized in Table 123.

Table 123: Mean (%CV) d-Nebivolol Pharmacokinetic Parameters in Three Healthy Male and Female Poor Metabolizers Following a Daily Oral Dose of 10mg Nebivolol HCL for Ten Days Alone or Concomitantly with a Single Oral Dose of 40mg Furosemide

Parameter	Treatment A Nebivolol	Treatment C Nebivolol + Furosemide	LSMEAN* Ratio (C/A)	90% Confidence Interval
CPEAK (ng/mL)	9.939 (11.6)	10.991 (13.8)	1.10	106% - 115%
CSS (ng/mL)	6.796 (11.5)	7.195 (16.9)	1.05	96% - 116%
AUCTAU (ng•hr/mL)	163.1 (11.5)	172.7 (16.9)	1.05	96% - 116%
CTROUGH (ng/mL)	4.446 (15.9)	4.258 (18.7)	0.95	85% - 107%
CMIN (ng/mL)	4.207 (18.5)	4.194 (18.5)	-	-
KEL (hr ⁻¹)	0.039 (19.8)	0.035 (6.0)	-	-
HALF (hr)	18.1 (17.9)	19.8 (5.8)	-	-
CL/F (L/hr)	31 (11.2)	29 (15.6)	-	-
Vd/F (L)	800 (13.8)	838 (13.2)	-	-
TPEAK (hr)	5.667 (10.2)	5.667 (10.2)	-	-
TMIN (hr)	16.083 (85.3)	8.000 (173.2)	-	-

There was a statistically significant increase in d-nebivolol CPEAK when furosemide was present, however, this increase is not clinically relevant based on the no-effect confidence interval range. There was no change in any of the other d-nebivolol PK measures in the presence of furosemide.

l- Nebivolol

The mean concentration versus time profiles for l- nebivolol in EM and PM subjects are illustrated graphically in Figure 2.

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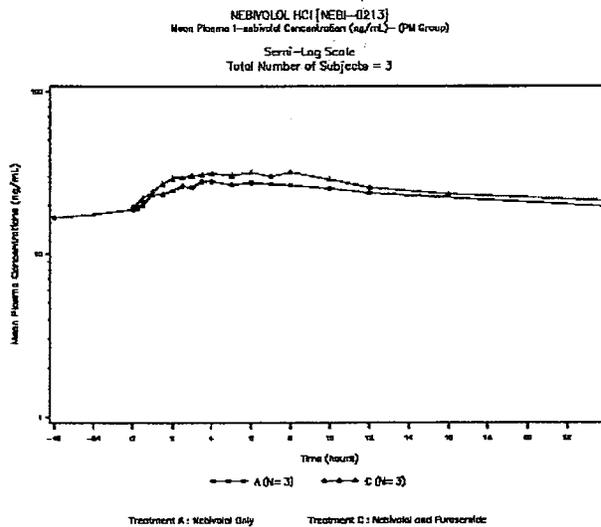
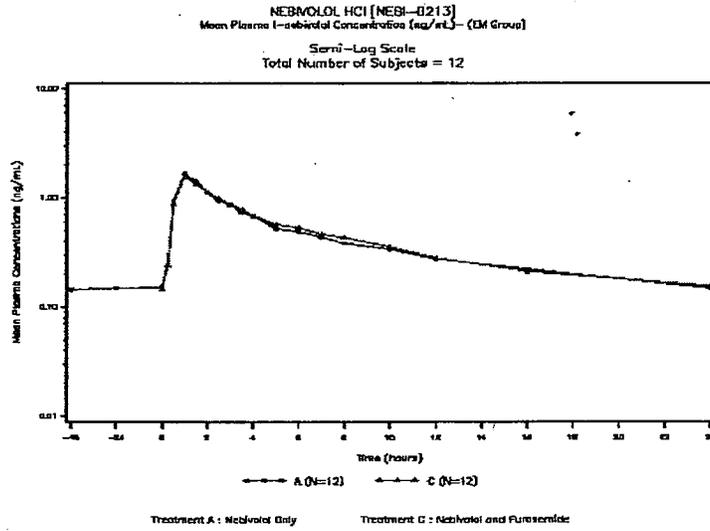


Figure 81: Plasma concentration-time profile of l-nebivolol in EMs (upper panel) and PMs (lower panel)

Pharmacokinetic data for l- nebivolol in EM subjects and PM subjects are presented in Table 124 and Table 125.

From ANOVA analysis of pharmacokinetic parameters, apart from increased CPEAK in PMs, concomitant administration of furosemide with nebivolol did not produce statistically significant changes in estimates for l- nebivolol in EM or PM subjects, (Table

124 and Table 125). The increase in CPEAK does not appear clinically significant as the 90 % confidence interval is just outside the no-effect range.

Table 124: Mean (%CV) I-Nebivolol Pharmacokinetic Parameters in Twelve Healthy Male and Female Extensive Metabolizers Following a Daily Oral Dose of 10mg Nebivolol HCL for Ten Days Alone or Concomitantly with a Single Oral Dose of 40mg Furosemide

Parameter	Treatment A Nebivolol	Treatment C Nebivolol + Furosemide	LSMEAN Ratio (C/A)	90% Confidence Interval
CPEAK (ng/mL)	1.735 (43.6)	1.649 (28.0)	0.98	88% - 110%
CSS (ng/mL)	0.409 (27.9)	0.415 (24.9)	1.02	98% - 106%
AUCTAU (ng•hr/mL)	9.8 (27.9)	10.0 (24.9)	1.02	98% - 106%
CTROUGH (ng/mL)	0.151 (28.9)	0.147 (23.8)	0.98	94% - 103%
CMIN (ng/mL)	0.144 (25.7)	0.143 (24.4)	-	-
KEL (hr ⁻¹)	0.058 (14.6)	0.052 (26.9)	-	-
HALF (hr)	12.2 (14.6)	14.5 (38.1)	-	-
CL/F (L/hr)	540 (23.9)	526 (20.6)	-	-
Vd/F(L)	9656 (34.9)	11,314 (51.7)	-	-
TPEAK (hr)	1.125 (33.5)	1.125 (27.6)	-	-
TMIN (hr)	10.000 (123.6)	8.021 (147.1)	-	-

Table 125: Mean (%CV) I-Nebivolol Pharmacokinetic Parameters in Three Healthy Male and Female Poor Metabolizers Following a Daily Oral Dose of 10mg Nebivolol HCL for Ten Days Alone or Concomitantly with a Single Oral Dose of 40mg Furosemide

Parameter	Treatment A Nebivolol	Treatment C Nebivolol + Furosemide	LSMEAN* Ratio (C/A)	90% Confidence Interval**
CPEAK (ng/mL)	28.438 (10.4)	32.803 (17.6)	1.15	102% - 129%
CSS (ng/mL)	23.565 (11.6)	26.026 (18.2)	1.10	98% - 123%
AUCTAU (ng•hr/mL)	565.6 (11.6)	624.6 (18.2)	1.10	98% - 123%
CTROUGH (ng/mL)	18.815 (13.8)	19.562 (17.5)	1.04	96% - 111%
CMIN (ng/mL)	18.696 (14.9)	19.562 (17.5)	-	-
KEL (hr ⁻¹)	0.018 (28.0)	0.016 (10.3)	-	-
HALF (hr)	39.6 (27.4)	42.9 (10.9)	-	-
CL/F (L/hr)	9 (11.7)	8 (17.5)	-	-
Vd/F (L)	499 (16.1)	500 (7.9)	-	-
TPEAK (hr)	5.333 (43.3)	5.333 (43.3)	-	-
TMIN (hr)	0.083 (173.2)	0.000 (n/a)	-	-

Furosemide

The mean concentration versus time profile for furosemide in all subjects (n= 15) is illustrated graphically in Figure 82.

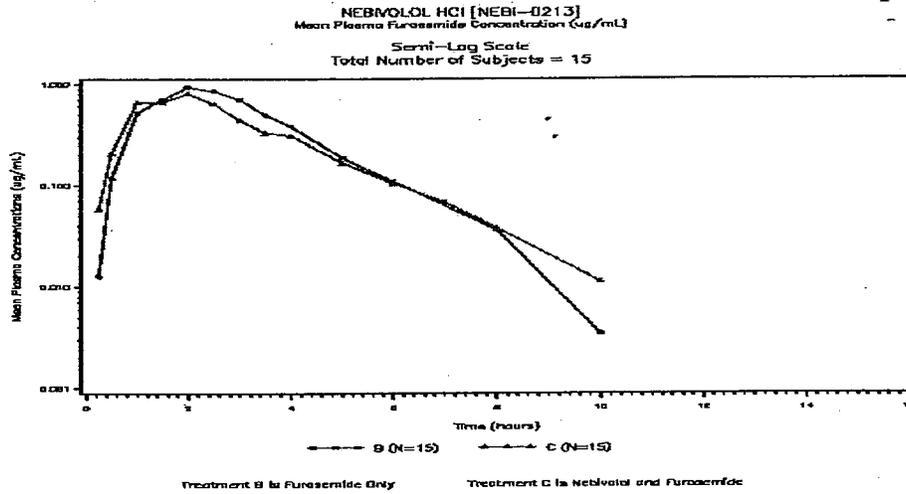


Figure 82: Furosemide Plasma concentration-time profile in all subjects

Pharmacokinetic data for furosemide in all subjects are presented in Table 126.

Table 126: Mean (%CV) Furosemide Pharmacokinetic Parameters in Fifteen Healthy Male and Female Subjects Following a Daily Oral Dose of 10mg Nebivolol HCL for Ten Days Alone or Concomitantly with a Single Oral Dose of 40mg Furosemide

Parameter	Treatment B Furosemide	Treatment C Nebivolol + Furosemide	LSMEAN Ratio (C/B)	90% Confidence Interval
CPEAK (µg/mL)	1.185 (38.6)	1.078 (34.0)	0.95	76% - 120%
AUCL (µg·hr/mL)	2.8 (39.2)	2.5 (29.6)	0.93	76% - 114%
AUCI (µg·hr/mL)	3.0 (36.9)	2.6 (27.6)	0.94	78% - 113%
KEL (hr ⁻¹)	0.504 (22.9)	0.408 (27.9)	-	-
HALF (hr)	1.5 (26.7)	1.8 (28.6)	-	-
CL/F (L/hr)	17 (67.6)	16 (27.9)	-	-
Vd/F (L)	35 (69.4)	44 (46.3)	-	-

ANOVA analyses of pharmacokinetic parameter estimates indicate that concomitant administration of furosemide with nebivolol produced no change in pharmacokinetic parameter estimates for furosemide, with the exception of KEL. The 90% confidence intervals for CPEAK, AUCL and AUCI were slightly outside of the 80% to 125% no-effect range. There was a 20% decrease in the mean KEL of furosemide when nebivolol was present, however this increase in KEL does not appear to be clinically significant.

Applicant’s Safety Analysis

Laboratory, vital sign and ECG monitoring indicated no safety risk associated with oral dosing of 10mg nebivolol HCL tablets alone or concomitantly with 40mg (1 x 40mg) furosemide tablets.

Conclusion

Coadministration of furosemide and nebivolol does not lead to drug-drug interactions that would affect the clinical pharmacokinetic profile or the safety of either nebivolol HCL or furosemide..

Labeling Recommendation (Discussion)

In general the applicant's labeling language is acceptable as it reports the study findings: no pharmacokinetic (PK) interactions are observed between nebivolol and furosemide. It is noted that there were statistically significant increases in CPEAK (both d- and l-nebivolol) in poor metabolizers, but the increases were $\leq 15\%$ and do not appear clinically relevant. Furthermore, these increases were obtained in a relatively small number of subjects (n=3). To be more concise the labeling can be modified to indicate that no clinically significant PK interactions were observed between nebivolol and furosemide.

Appears This Way
On Original

4.2.23 A Phase I Open-Label Multiple-Dose Study Assessing the Pharmacokinetic Interaction Between Spironolactone and Nebivolol HCl in Healthy Volunteers (NEBI-0214)

INVESTIGATORS/ STUDY SITE	James D. Carlson, Pharm.D.
STUDY PERIOD	November 20, 2002 to December 24, 2002

Summary of Drug-Drug interaction Potential for Study Rationale

	Spironolactone	Nebivolol
Indication/Mechanism of Action	Diuretic and antihypertensive drug. Given alone or with β -blockers to reduce edema and peripheral vascular resistance in cardiac failure	Proposed for treatment of hypertension
Metabolites (Activity)	7 α -thiomethyl-spironolactone, and other sulfur-containing metabolites. Metabolites and parent compound believed to be responsible for activity (Gardiner, 1989)	Several metabolites including, glucuronides (major), hydroxy and oxidative metabolites
Metabolic Pathway	rapidly and extensively metabolized by carboxyl esterase and glutathione-S-transferase;	CYP2D6 substrate
CYP Inhibitory Potential	None reported	Low potential to inhibit CYP
Highest Recommended Dose/Studied Dose	Individualized therapy, typical doses range from 25 to 200 mg (Package Insert, 2003). Given as single or divided dose.	10 mg QD

Study Objective

To determine if co-administration of spironolactone with nebivolol HCL altered the steady-state pharmacokinetics of either nebivolol or spironolactone

Reviewer's Note on Study Objective

The initial objective of this study was to determine if co-administration of spironolactone with nebivolol HCL altered the steady-state pharmacokinetics of either nebivolol or spironolactone. Plasma samples reserved for spironolactone determinations could not be analyzed.

Study Design

Subjects: Sixteen, non-smoking, adult, male and female healthy volunteers between the ages of 18 to 63 were accepted into the clinical phase of this study.

Subjects were genotyped to determine their CYP2D6 metabolizing status and were assigned to the following treatments, based upon the randomization scheme.

- TREATMENT A: 10mg (1 x 10mg) nebivolol QD for Ten Days
- TREATMENT B: 25mg (1 x 25mg) spironolactone QD for Ten Days
- TREATMENT AB*: 10mg (1 x 10mg) nebivolol and 25mg (1 x 25mg) Spironolactone QD for Ten Days

Drug Formulations

- Nebivolol HCL Tablets, 10mg, Mylan Pharmaceuticals Inc., Lot # R1H1182
- Spironolactone Tablets, USP 25mg Mylan Pharmaceuticals Inc., Lot # 1K0300

Blood Sampling

- Days 1, 8 and 9: predose blood samples were collected
- Day 10: predose and 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 16 and 24 hours.
- Days 18 and 19: predose samples
- Day 20 predose and 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 16 and 24 hours post dose.
- Day 28 and Day 29: predose sample
- Day 30: predose and 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 16, 24, 48, 72 and 96 hours post dose.

Analytical Methods

HPLC with tandem mass spectrometric detection was used to determine the concentrations of *d*-neбиволol and *l*-neбиволol in human plasma (heparin. Assay performance was acceptable as shown in Table 127.

Table 127: Assay Characteristics for d- and l-Nebivolol

Parameter	Measure	Reviewer Comment
	Assay for Extensive Metabolizers (Curve III)	
Linearity	linear from 0.04ng/mL to 3.0ng/mL	Satisfactory
CV (%) Between day Precision	d-neбиволol ≤ 7.0 % l-neбиволol ≤ 5.9 %	Satisfactory
Relative Bias Between day Accuracy	d-neбиволol between -5.8 % and 6.2 % l-neбиволol between -5.9% and 5.5 %	Satisfactory
LLOQ	0.04ng/mL	Satisfactory
Specificity	Chromatograms provided demonstrated assay specificity	Satisfactory
	Assay for Poor Metabolizers (Curve II)	
Linearity	linear from 0.2ng/mL to 15ng/mL	Satisfactory
CV : Between day Precision	d-neбиволol ≤ 5.1 % l-neбиволol ≤ 6.6 %	Satisfactory
Relative Bias Between day Accuracy	d-neбиволol between -6.5% and 6.1% l-neбиволol between -6.7% and 6.1%	Satisfactory
LLOQ	0.2 ng/mL	Satisfactory
Specificity	Chromatograms provided demonstrated assay specificity	Satisfactory

Spironolactone Assay

According to the applicant, samples for spironolactone analyses were inadvertently thawed during shipping and remained at ambient temperature until received by MDS Pharma (Québec, Canada). The shipping container was opened by the courier and all dry ice in the container sublimed. Therefore, plasma samples were not analyzed, as it was impossible to determine sample storage conditions during shipping.

Pharmacokinetics

Steady-state pharmacokinetic parameters for *d*-neбиволol and *l*-neбиволol were calculated

using noncompartmental techniques. The following pharmacokinetic measures for *d*- and *l*-neбиволol were generated: CPEAK and TPEAK (Day 10, 20 or 30), AUC_t, CMIN, and TMIN, CTROUGH, CSS, KEL, HALF, CL/F, and Vd/F.

Statistics

The occurrence of a drug-drug interaction was evaluated by standard pharmacokinetic-statistical methods. The test treatment was nebivolol + spironolactone and the reference treatment was nebivolol alone.

Results and Discussion

Pharmacokinetic Analyses

Data are presented for fifteen subjects (11 EM and 4 PM), except where indicated. Steady-state plasma concentrations were achieved by Day 10, Day 20 and/or Day 30 (depending on the randomization schedule) for *d*-neбиволol and *l*-neбиволol.

d-neбиволol

The mean concentration versus time profiles for *d*-neбиволol in EM and PM are depicted in Figure 83.

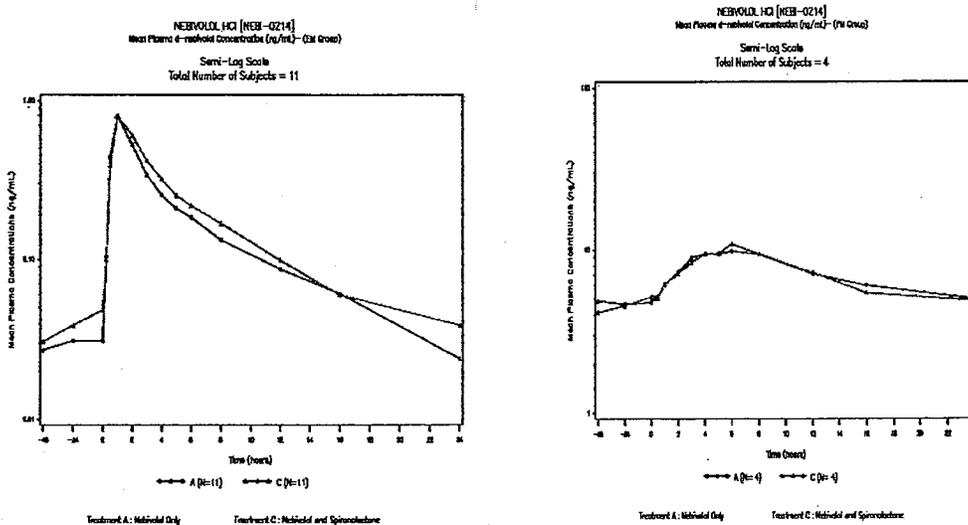


Figure 83: Plasma Concentration time profile for *d*-neбиволol in EM and PM

Pharmacokinetic data for *d*-neбиволol in EMs are presented in Table 128. Based upon ANOVA analysis, concomitant administration of spironolactone with nebivolol did not produce statistically significant changes in primary pharmacokinetic measures (AUC and CPEAK) estimated for *d*-neбиволol in EM subjects.

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Table 128: Mean (%CV) *d*-Nebivolol Pharmacokinetic Parameters in Eleven Healthy Male and Female Extensive Metabolizers Following a Daily Oral Dose of 10mg Nebivolol HCL for Ten Days Alone or Concomitantly with a Daily Oral Dose of 25mg Spironolactone

Parameter	Treatment A Nebivolol	Treatment C Nebivolol + Spironolactone	LSMEANS Ratio (C/A)	90% Confidence Interval
CPEAK (ng/mL)	0.803 (43.7)	0.803 (36.8)	1.03	96% - 110%
CSS (ng/mL)	0.150 (40.5)	0.171 (37.7)	1.14	100% - 129%
AUCTAU [(ng·hr)/mL]	3.604 (40.5)	4.113 (37.7)	1.14	100% - 129%
CTROUGH (ng/mL)	0.031 (108.0)	0.048 (61.7)	1.18	102% - 137%
CMIN (ng/mL)	0.023 (117.9)	0.038 (67.5)	—	—
KEL (hr ⁻¹)	0.084 (35.7)	0.090 (39.6)	—	—
HALF (hr)	9.1 (31.1)	8.6 (29.1)	—	—
CL/F (L/hr)	1622 (43.3)	1451 (53.4)	—	—
Vd/F (L)	19,669 (32.0)	16,458 (33.5)	—	—
TPEAK (hr)	0.961 (16.1)	1.091 (27.6)	—	—
TMIN (hr)	8.727 (138.7)	15.273 (79.3)	—	—

Pharmacokinetic data for *d*-nebivolol in PMs are presented in Table 129.

Table 129: Mean (%CV) *d*-Nebivolol Pharmacokinetic Parameters in Four Healthy Male and Female Poor Metabolizers Following a Daily Oral Dose of 10mg Nebivolol HCL for Ten Days Alone or Concomitantly with a Daily Oral Dose of 25mg Spironolactone

Parameter	Treatment A Nebivolol	Treatment C Nebivolol + Spironolactone	LSMEANS* Ratio (C/A)	90% Confidence Interval**
CPEAK (ng/mL)	9.942 (12.3)	10.857 (12.6)	1.09	100% - 120%
CSS (ng/mL)	7.127 (12.1)	7.023 (12.5)	0.99	95% - 102%
AUCTAU [(ng·hr)/mL]	171.1 (12.1)	168.6 (12.5)	0.99	95% - 102%
CTROUGH (ng/mL)	4.754 (11.3)	5.133 (13.9)	1.08	100% - 116%
CMIN (ng/mL)	4.698 (12.4)	4.692 (13.4)	—	—
KEL (hr ⁻¹)	0.033 (19.5)	0.039 (18.3)	—	—
HALF (hr)	21.6 (24.2)	18.0 (18.9)	—	—
CL/F (L/hr)	30 (12.7)	30 (12.3)	—	—
Vd/F (L)	908 (16.1)	731 (9.0) _a	—	—
TPEAK (hr)	5.500 (18.2)	6.000 (00.0)	—	—
TMIN (hr)	6.125 (194.6)	16.063 (69.7)	—	—

Based upon ANOVA analysis, concomitant administration of spironolactone with nebivolol did not produce statistically significant changes in primary pharmacokinetic measures (AUC and CPEAK) estimated for *d*-nebivolol in PM subjects.

l-nebivolol

The mean concentration versus time profiles for *l*-neбиволol in EM and PM are depicted in Figure 84.

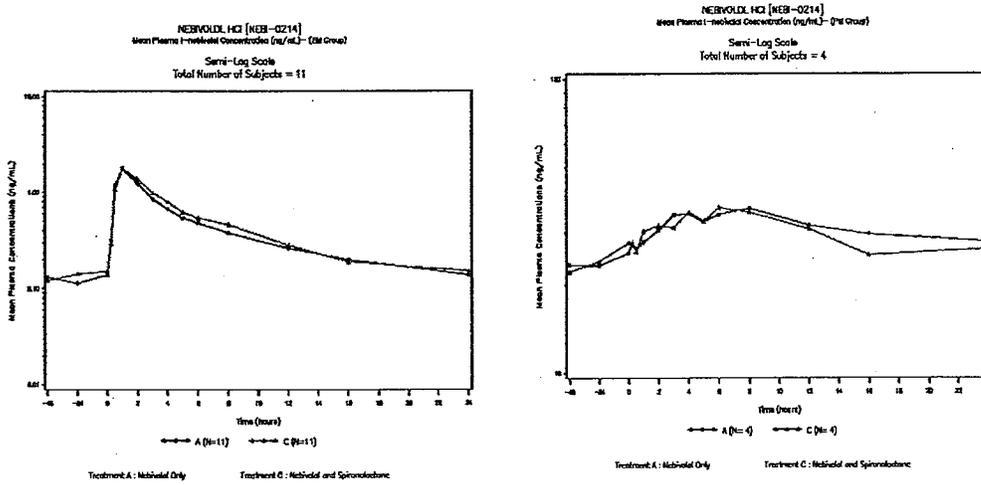


Figure 84: Plasma Concentration time profile for *l*-neбиволol in EM and PM

Pharmacokinetic data for *l*-neбиволol in EMs are presented in Table 130.

Table 130: Mean (%CV) *l*-Nebivolol Pharmacokinetic Parameters in Eleven Healthy Male and Female Extensive Metabolizers Following a Daily Oral Dose of 10mg Nebivolol HCL for Ten Days Alone or Concomitantly with a Daily Oral Dose of 25mg Spironolactone

Parameter	Treatment A Nebivolol	Treatment C Nebivolol + Spironolactone	LSMEANS* Ratio (C/A)	90% Confidence Interval**
CPEAK (ng/mL)	1.884 (51.6)	1.786 (35.0)	1.01	89% - 115%
CSS (ng/mL)	0.409 (30.3)	0.444 (29.6)	1.09	101% - 118%
AUCTAU [(ng•hr)/mL]	9.804 (30.3)	10.65 (29.6)	1.09	101% - 118%
CTROUGH (ng/mL)	0.138 (24.7)	0.152 (23.2)	1.10	102% - 118%
CMIN (ng/mL)	0.132 (18.7)	0.147 (22.7)	—	—
KEL (hr ⁻¹)	0.055 (20.6)	0.058 (24.1)	—	—
HALF (hr)	13.2 (22.7)	12.7 (25.0)	—	—
CL/F (L/hr)	563 (36.3)	516 (34.7)	—	—
Vd/F (L)	10,948 (51.0)	9452 (43.6)	—	—
TPEAK (hr)	1.052 (33.3)	1.091 (27.6)	—	—
TMIN (hr)	6.545 (171.3)	10.909 (115.0)	—	—

Based upon ANOVA analysis, concomitant administration of spironolactone with neбиволol increased *l*-neбиволol AUC, CSS and CTROUGH. However, these exposure increases are not clinically relevant based on the confidence interval range.

Pharmacokinetic data for *l*-neбиволol in PMs are presented in Table 131.

Table 131: Mean (%CV) *l*-Nebivolol Pharmacokinetic Parameters in Four Healthy Male and Female Poor Metabolizers Following a Daily Oral Dose of 10mg Nebivolol HCL for Ten Days Alone or Concomitantly with a Daily Oral Dose of 25mg Spironolactone

Parameter	Treatment A Nebivolol	Treatment C Nebivolol + Spironolactone	LSMEANS* Ratio (C/A)	90% Confidence Interval**
CPEAK (ng/mL)	36.926 (20.6)	36.473 (12.1)	1.00	82% - 121%
CSS (ng/mL)	31.268 (18.4)	29.738 (12.7)	0.96	82% - 111%
AUCTAU [(ng·hr)/mL]	750.4 (18.4)	713.7 (12.7)	0.96	82% - 111%
CTROUGH (ng/mL)	25.581 (23.6)	27.641 (16.9)	1.09	87% - 136%
CMIN (ng/mL)	25.010 (20.9)	23.969 (15.2)	—	—
KEL (hr ⁻¹)	0.016	0.011	—	—
HALF (hr)	48.2	64.6	—	—
CL/F (L/hr)	7 (15.6)	7 (13.8)	—	—
Vd/F (L)	516	611	—	—
TPEAK (hr)	4.750 (46.7)	6.500 (15.4)	—	—
TMIN (hr)	0.375 (127.7)	16.750 (59.2)	—	—

Based upon ANOVA analysis, concomitant administration of spironolactone with nebivolol did not alter *l*-nebivolol exposure. Most of the confidence intervals were within the no-effect range.

Applicant's Safety Analyses

Laboratory, vital sign and ECG monitoring indicated no safety risk associated with oral dosing of 10mg nebivolol HCL tablets alone or concomitantly with 25mg (1 x 25mg) spironolactone tablets. Regardless of CYP2D6 metabolizing status, once-daily administration of nebivolol HCL (10 mg) alone or in combination with spironolactone (25 mg) was well tolerated in healthy adult volunteers. Adverse events related to nebivolol HCL administration were mild in severity.

CONCLUSIONS

Coadministration of spironolactone with nebivolol does not produce clinically significant changes in nebivolol pharmacokinetics. Nebivolol (*d*-nebivolol) exposure increased by a maximum of 18 % (CTROUGH) in EMs.

Labeling Recommendations

There are no drug interactions that would affect the clinical pharmacokinetic profile or the safety of nebivolol when co-administered with spironolactone. The applicant's labeling proposal is acceptable: description of study and study findings (no pharmacokinetic interaction between nebivolol and spironolactone).

Appears This Way
On Original

4.2.24 A Phase I Open-Label Multiple-Dose Study of the Effect of Nebivolol HCl on the Pharmacokinetics of Spironolactone in Healthy Volunteers

INVESTIGATORS/ STUDY SITE	James D. Carlson, Pharm.D.
STUDY PERIOD	January 7, 2004 – April 5, 2004

Summary of Drug-Drug interaction Potential (Study Rationale)

	Spironolactone	Nebivolol
Indication/Mechanism of Action	Diuretic and antihypertensive drug. Given alone or with β -blockers to reduce edema and peripheral vascular resistance in cardiac failure	Proposed for treatment of hypertension
Metabolites (Activity)	7 α -thiomethyl-spironolactone, and other sulfur-containing metabolites. Metabolites and parent compound believed to be responsible for activity (Gardiner, 1989)	Several metabolites including, glucuronides (major), hydroxy and oxidative metabolites
Metabolic Pathway	rapidly and extensively metabolized by carboxyl esterase and glutathione-S-transferase;	CYP2D6 substrate
CYP Inhibitory Potential	None reported	Low potential to inhibit CYP
Highest Recommended Dose/Studied Dose	Individualized therapy, typical doses range from 25 to 200 mg (Package Insert, 2003). Given as single or divided dose.	10 mg QD

Study Objective

To determine if co-administration of spironolactone with nebivolol HCL altered the steady-state pharmacokinetics of spironolactone.

Study Design

Thirty-six non-smoking, adult, male and female healthy volunteers between the ages of 19 to 58 were enrolled. Subjects were genotyped to determine their CYP2D6 metabolizing status and were assigned to the following treatments, based upon the randomization scheme.

- Treatment A (Days 1 – 10): 25mg (1 x 25mg) spironolactone QD
- Treatment B (Days 11 – 20): 25 mg spironolactone + 10 mg (1 x 10mg) nebivolol QD.

Drug Formulations

- Nebivolol HCL Tablets, 10mg, Mylan Pharmaceuticals Inc. Lot # R1H1182
- Spironolactone Tablets, USP 25mg Mylan Pharmaceuticals Inc. Lot # 1K0300

Blood Sampling

- Days 1, 8, 9, 18 and 19: predose blood samples were collected
- Day 10 and 20: predose and 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 16 and 24 hours.

Analytical Methods

Samples were assayed for spironolactone and its two major metabolites, canrenone and 7 α -thiomethyl spironolactone by HPLC with tandem mass spectrometric detection. Assay performance was acceptable as shown in Table 132.

Table 132: Spironolactone Assay Characteristics

Parameter	Measure			Reviewer Comment
	Spironolactone	canrenone	7 α -thiomethyl spironolactone	
Linearity (ng/mL)	2.00 – 60.0	2.00 – 120	5.00 – 200	Satisfactory
CV, Between day Precision	≤ 7.2 %	≤ 8.7 %	≤ 6.0 %	Satisfactory
Relative Bias Accuracy Between Day	Between -3.5 and 5.4 % of nominal concentration	Between -5.7 and 13 % of nominal concentration	Between -3.2 and 10.6 % of nominal concentration	Satisfactory
Specificity	Chromatograms provided demonstrate assay specificity			Satisfactory

Pharmacokinetics

Steady-state pharmacokinetic parameters for spironolactone, canrenone and 7 α -thiomethyl spironolactone were calculated using noncompartmental techniques. The following pharmacokinetic measures for spironolactone and its metabolites were estimated: CPEAK, TEPAK, AUC τ , CMIN and TMIN, CSS, KEL, t $_{1/2}$, CL/F, and Vd/F

Statistics

Drug-drug interactions were evaluated by standard pharmaco-statistical procedures. The test treatment was spironolactone + nebivolol and the reference treatment was spironolactone alone.

RESULTS AND DISCUSSION

Spironolactone Pharmacokinetics

Data are presented for thirty-five subjects, except where indicated. The plasma concentration-time profiles of spironolactone and its major metabolites appear similar in the presence and absence of nebivolol (Figure 85).

Based upon ANOVA analysis, concomitant administration of spironolactone with nebivolol did not produce statistically significant changes in primary pharmacokinetic parameters estimated for spironolactone (Table 133)

Pharmacokinetic data for canrenone are presented in Table 134. Based on ANOVA analysis, concomitant administration of spironolactone with nebivolol produced statistically significant ($p < 0.05$) changes in CPEAK, AUCTAU, CSS and AUCTAU and CSS for canrenone; however, the changes are not clinically significant because they fall within the no-effect confidence interval boundary (80 - 125).

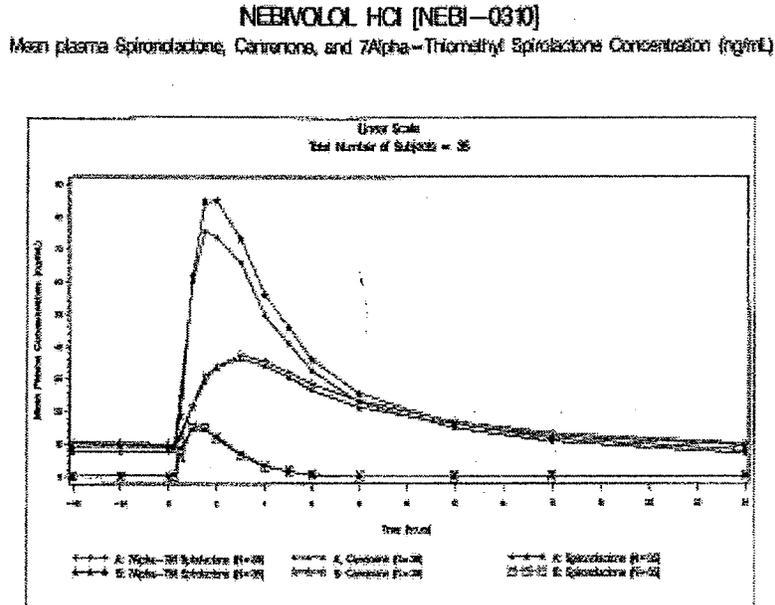


Figure 85: Plasma Concentration-time Profiles for Spironolactone, Canrenone, and 7 α -Thiomethyl Spironolactone in Healthy Subjects (A- spironolactone alone and B- spironolactone + nebivolol)

Table 133: Mean (%CV) Spironolactone Pharmacokinetic Parameters in Thirty-five Healthy Male and Female Volunteers Following a Daily Oral Dose of 25mg Spironolactone for Ten Days Alone or Concomitantly with a Daily Oral Dose of 10mg Nebivolol HCL

Parameter	Treatment A Spironolactone (Day 10)	Treatment B Nebivolol + Spironolactone (Day 20)	LSMEANS Ratio (B/A)	90% Confidence Interval
CPEAK (ng/mL)	17.61 (42.2)	19.03 (44.1)	1.06	95% - 119%
CSS (ng/mL)	1.644 (44.8)	1.598 (44.8)	0.95	90% - 102%
AUCTAU [(ng·hr)/mL]	39.46 (44.8)	38.36 (44.8)	0.95	90% - 102%
KEL (hr ⁻¹)	0.654 (32.2)	0.692 (29.1)	-	-
HALF (hr)	1.27 (58.1)	1.14(49.5)	-	-
CL/F (L/hr)	771.0 (46.2)	838.3 (56.5)	-	-
Vd/F (L)	1,354 (106.8)	1,232 (123.6)	-	-
TPEAK (hr)	1.47 (40.3)	1.53 (50.8)	-	-

Table 134: Mean (%CV) Canrenone Pharmacokinetic Parameters in Thirty-five Healthy Male and Female Volunteers Following a Daily Oral Dose of 25mg Spironolactone for Ten Days Alone or Concomitantly with a Daily Oral Dose of 25mg Nebivolol HCL

Parameter	Treatment A Spironolactone (Day 10)	Treatment B Nebivolol + Spironolactone (Day 20)	LSMEANS Ratio (B/A)	90% Confidence Interval
CPEAK (ng/mL)	38.74 (26.8)	40.80 (30.3)	1.04	100% - 109%
CSS (ng/mL)	18.38 (29.9)	19.37 (29.8)	1.05	103% - 108%
AUCTAU [(ng·hr)/mL]	441.0 (29.9)	464.8 (29.8)	1.05	103% - 108%
CTROUGH (ng/mL)	9.828 (42.6)	9.944 (37.4)	1.03	99% - 107%
CMIN (ng/mL)	9.107 (44.7)	9.141 (41.2)	—	—
TPEAK (hr)	2.87 (32.0)	2.97 (32.6)	—	—
TMIN (hr)	14.4 (82.3)	7.01 (155)	—	—

Pharmacokinetic data for 7 α -thiomethyl spironolactone are presented in Table 135.

Table 135: Mean (%CV) 7 α -Thiomethyl Spironolactone Pharmacokinetic Parameters in Thirty-five Healthy Male and Female Subjects Following a Daily Oral Dose of 25mg Spironolactone for Ten Days Alone or Concomitantly with a Daily Oral Dose of 10mg Nebivolol HCL

Parameter	Treatment A Nebivolol (Day 10)	Treatment B Nebivolol + Spironolactone (Day 20)	LSMEANS Ratio (B/A)	90% Confidence Interval
CPEAK (ng/mL)	85.86 (38.9)	99.87 (32.5)	1.19	110% - 129%
CSS (ng/mL)	23.37 (45.4)	25.85 (37.1)	1.14	107% - 120%
AUCTAU [(ng·hr)/mL]	560.9 (45.4)	620.3 (37.1)	1.14	107% - 120%
CTROUGH (ng/mL)	7.597 (88.3)	8.896 (69.3)	1.09	99% - 120%
CMIN (ng/mL)	6.447 (98.4)	7.523 (79.5)	—	—
TPEAK (hr)	1.94 (39.9)	2.11 (39.4)	—	—
TMIN (hr)	13.0 (93.0)	10.4 (116)	—	—

Based upon ANOVA analysis, concomitant administration of spironolactone with nebivolol produced statistically significant ($p < 0.05$) changes in plasma concentrations (CPEAK, AUCTAU, CTROUGH, CSS, CPEAK, AUCTAU and CSS) of 7 α -thiomethyl spironolactone. However the majority of the primary parameters fall within the no effect boundary; thus the changes do not appear to be clinically relevant.

Applicant's Safety Summary

Laboratory, vital sign and ECG monitoring indicated no safety risk associated with oral dosing of 10mg nebivolol HCL tablets concomitantly with 25mg (1 x 25mg) spironolactone tablets. Sixteen subjects were enrolled in and fifteen completed this study. Subject No. 8 elected to withdraw on study Day 1. There were no serious or life threatening adverse events reported for this study.

Conclusion

There were no drug interactions that would affect the clinical pharmacokinetic profile or the safety of spironolactone when co-administered with nebivolol.

Labeling Recommendations

No dosage adjustment is required for spironolactone-nebivolol coadministration because exposure changes (increases) in spironolactone and its two major metabolites were not significant. The findings from the study should be included in the label.

Reviewer's Proposed Labeling:

Concomitant administration of spironolactone 25 mg once daily) with nebivolol (10 mg once daily) for 10 days did not produce clinically significant changes in spironolactone exposure or the exposure of spironolactone's major metabolites.

Reviewer's Note

The applicant did not propose labeling for the spironolactone study because in the original study, spironolactone plasma samples were not analyzed.

Appears This Way
On Original

4.2.25 A Phase I Open-Label Multiple-Dose Study Assessing the Pharmacokinetic Interaction Between Ramipril and Nebivolol HCl in Healthy Volunteers (Protocol NEBI-0220)

INVESTIGATORS/ Study Site	James D. Carlson, Pharm.D., _____
STUDY PERIOD	October 9, 2002 to November 14, 2002

Summary of Drug-Drug Interaction Potential (Study Rationale)

	Ramipril	Nebivolol
Mechanism of Action/ Typical Use	non-sulphydryl ACE-inhibitor antihypertensive	Proposed for treatment of hypertension
Metabolites (Activity)	Ramiprilat is major metabolite (~ 6 x more potent than ramipril). Other metabolites: diketopiperazine ester, diketopiperazine acid, and ramipril and ramiprilat glucuronide.	Several metabolites including, glucuronides (major), hydroxy and oxidative metabolites
Metabolic Pathway	Ramipril is converted to ramiprilat by hepatic cleavage of the ester group; ramipril is almost completely metabolized into ramiprilat	CYP2D6 substrate
CYP Inhibitory Potential	None reported	Low potential to inhibit CYP
Interaction Pathway/Mechanism	None expected	None clearly identified.
Highest Recommended Dose/Studied Dose	Individualized dosage. Initial oral dose in patients not on diuretic therapy is 2.5 mg ramipril QD. Typical dosage range: 2.5 to 20 mg per day as a single dose or in two equally divided doses.	Individualized, initial 5 mg QD but anticipated 10 mg QD

STUDY OBJECTIVE

To determine if co-administration of ramipril with nebivolol HCL alters the pharmacokinetics of nebivolol or ramipril.

Study Conduct

Fifteen, non-smoking, adult, male and female volunteers participated in the study. Subjects were genotyped to determine their CYP2D6 metabolizing status and were randomized into two groups, Group 1 and Group 2, consisting of seven and eight subjects, respectively. Group 1 consisted of 6 EM and 1 PM subject, Group 2 consisted of 6 EM and 2 PM subjects. Subjects were assigned by treatment and according to the tabulated randomization schedule (below).

Group	Treatment Sequence	Subject	CYP2D6 Metabolic Status
1	A AB B	2, 4, 5, 8, 10, 12	EM
		15, 16	PM
2	B AB A	1, 3, 6, 7, 9, 11	EM
		13, 14	PM

TREATMENT A: 10mg (1 x 10mg) Nebivolol QD for 10 days
TREATMENT B: 5mg (1 x 5mg) Ramipril QD for 10 Days
TREATMENT AB: 10mg (1 x 10mg) Nebivolol and 5mg (1 x 5mg) Ramipril QD for 10 days

Group 1 received Treatment A (Days 1 to 10) followed by Treatment AB (Days 11 – 20) followed by Treatment B (Days 21 – 30). Group 2 subjects received Treatment B (Days 1 to 10) followed by Treatment AB (Days 11 – 20) and Treatment A (Days 21 to 30). All doses of nebivolol and ramipril were given with 240 mL of ambient temperature water. Treatments were given in the fasted state: subjects fasted at least 10hr prior to dosing until 4hr after dosing on Days 1, 10, 20 and 30. Standard meals were provided on the evenings prior to dosing and at 4 and 10hr after dosing on Days 1, 10, 20 and 30.

Subject Characteristics (n = 15)

Mean Age \pm SD: 33.1 \pm 14.9 years

Mean Weight \pm SD: 80.1 \pm 8.2 kg

Sex: 8 male and 7 female

Blood Sampling

For nebivolol

- Day 1, 8, 9, 18 and 19: pre-dose blood samples were collected
- Day 10: -0.5 (predose) and 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 16 and 24hr post dose.

For ramipril/ramiprilat

- Day 12: -0.5 and 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 16 and 24hr.
- Day 28 and 29: prior to dosing
- Day 30: -0.5 and 0.25, 0.5, 1, 2, 3, 4, 5, 6, 8, 12, 16, 24, 48, 72, 96, 120 and 144hr.

Formulations

- Nebivolol HCL Tablets, 10mg, Mylan Pharmaceuticals Inc., Lot # R1H1182
- Altace® (Ramipril) Capsules, 5mg Aventis Pharmaceuticals, Inc., Lot # 1037820

Analytical Method

d- and l-nebivolol

A high performance liquid chromatography with tandem mass spectrometric detection method was used to determine *d*-nebivolol and *l*-nebivolol concentrations in human plasma (heparin). Assay performance was acceptable as shown in Table 136.

Ramipril/Ramiprilat

A high performance liquid chromatography with tandem mass spectrometric detection method was used to determine ramipril/ramiprilat concentrations in human plasma (heparin). Assay performance was acceptable as shown in Table 137.

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Table 136: Assay Characteristics for d- and l-Nebivolol

Parameter	Measure	Reviewer Comment	
	Assay for Extensive Metabolizers (Curve III)		
Linearity	linear from 0.04ng/mL to 3.0ng/mL	Satisfactory	
CV : Between day Precision	d-nebivolol \leq 14%	l-nebivolol \leq 15%	Satisfactory
Relative Bias Between day Accuracy	d-nebivolol between -9.3% and 4.4%	l-nebivolol between -5.8% and 4.4%	Satisfactory
LLOQ	0.04ng/mL	Satisfactory	
Specificity		Satisfactory	
	Assay for Poor Metabolizers (Curve II)		
Linearity	linear from 0.2ng/mL to 15ng/mL	Satisfactory	
CV : Between day Precision	d-nebivolol \leq 7.3%	l-nebivolol \leq 9.5%	Satisfactory
Relative Bias Between day Accuracy	d-nebivolol between -6.1% and 5.2%	l-nebivolol between -6.0% and 6.4%	Satisfactory
LLOQ	0.2ng/mL	Satisfactory	
Specificity	Chromatograms indicate that assay was specific	Satisfactory	

Table 137: Assay Characteristics for ramipril/ramiprilat

Parameter	Measure	Reviewer Comment	
Linearity	linear from 0.500 to 250ng/mL for ramipril and ramiprilat	Satisfactory	
CV : Between day Precision	ramipril \leq 8.8%	ramiprilat \leq 7.9%	Satisfactory
Relative Bias Between day Accuracy	ramipril between -5.3% and 2.6%	ramiprilat between -5.9% and 4.0%	Satisfactory
LLOQ	0.500ng/mL for ramipril and ramiprilat	Satisfactory	
Specificity	Chromatograms indicate that assay was specific	Satisfactory	

Pharmacokinetics

Pharmacokinetic parameters for *d*-nebivolol, *l*-nebivolol, ramipril and ramiprilat were calculated using noncompartmental techniques. The following PK measures were determined: CPEAK, TPEAK (Day 10, Day 20 and Day 30). AUCTAU on Day 10 or CMIN, TMIN, on Day 10, Day 20 or Day 30. CTROUGH (prior to dosing on Day 10, Day 20 or Day 30), $CSS_{avg} = AUCTAU/\tau$, ($\tau = 24hr$ for Day 10 and Day 20 or 96hr for Day 30); (KEL) on Day 10, Day 20 or Day 30. THALF; $CL/F = Dose/AUCTAU$, $Vd/F = (CL/F)/KEL$.

Statistics

The nebivolol-ramipril drug-drug interaction was assessed using standard pharmacostatistical tests. The test treatment was nebivolol + ramipril and the reference treatments were nebivolol alone or ramipril alone.

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RESULTS AND DISCUSSION

Pharmacokinetic Analyses

Data are presented for fifteen subjects (12 EM and 3 PM), except where indicated. In general, steady-state plasma concentrations were achieved by Day 10, Day 20 and/or Day 30 for *d*-nebivolol and *l*-nebivolol for both EM and PM subjects and/or ramiprilat in EM. Data were insufficient for assessment of steady-state ramiprilat concentrations in PM subjects. There was no accumulation of ramipril.

***d*-Nebivolol**

The mean concentration versus time profiles for *d*-nebivolol in EM and PM are depicted in Figure 86.

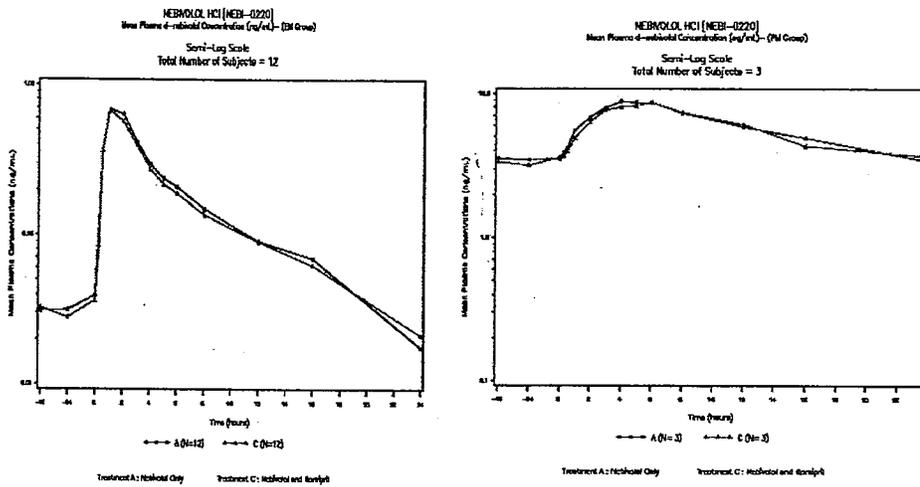


Figure 86: Concentration versus time profiles for *d*-nebivolol in EM and PM

Based on ANOVA analysis, concomitant administration of ramipril with nebivolol did not produce statistically significant changes in pharmacokinetic parameters for *d*-nebivolol in EM subjects (Table 138). The confidence intervals were slightly outside the no-effect boundaries for CPEAK and CTROUGH.

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Table 138: Mean (%CV) *d*-Nebivolol Pharmacokinetic Parameters in Twelve Healthy Male and Female Extensive Metabolizers Following a Daily Oral Dose of 10mg Nebivolol HCL for Ten Days Alone or Concomitantly with a Daily Oral Dose of 5mg Ramipril

Parameter	Treatment A	Treatment AB	LSMEANS*	90% Confidence
	Nebivolol	Nebivolol + Ramipril	Ratio (AB/A)	Interval
CPEAK (ng/mL)	0.818 (40.8)	0.765 (30.3)	0.96	77% - 119%
CSS (ng/mL)	0.161 (42.2)	0.151 (28.0)	0.97	90% - 103%
AUCTAU (ng•hr/mL)	3.874 (42.2)	3.617 (28.0)	0.97	90% - 103%
CTROUGH (ng/mL)	0.039 (86.2)	0.036 (78.7)	0.90	77% - 105%
CMIN (ng/mL)	0.017 (181.3)	0.021 (126.7)	-	-
KEL (hr ⁻¹)	0.099 (29.2)	0.086 (23.0)	-	-
HALF (hr)	7.678 (33.9)	8.540 (25.9)	-	-
CL/F (L/hr)	1454 (30.7)	1468 (23.3)	-	-
Vd/F ¹ (L)	15,082 (29.6)	17,759 (29.1)	-	-
TPEAK (hr)	1.750 (65.0)	1.375 (51.7)	-	-
TMIN (hr)	14.000 (88.3)	16.000 (73.9)	-	-

Concomitant administration of ramipril did not produce a statistically significant change in *d*-neбиволol PK measures for PMs (Table 139). Apart from CPEAK, the lower boundary of the confidence intervals for exposure measures was just outside the no-effect boundary. Overall, the changes in exposure do not appear to be clinically significant.

Table 139: Mean (%CV) *d*-Nebivolol Pharmacokinetic Parameters in Three Healthy Male and Female Poor Metabolizers Following a Daily Oral Dose of 10mg Nebivolol HCL for Ten Days Alone or Concomitantly with a Daily Oral Dose of 5mg Ramipril

Parameter	Treatment A	Treatment C	LSMEANS*	90% Confidence
	Nebivolol	Nebivolol + Ramipril	Ratio (C/A)	Interval
CPEAK (ng/mL)	9.277 (15.5)	8.789 (28.7)	0.93	69% - 125%
CSS (ng/mL)	5.890 (18.4)	5.852 (28.8)	0.97	79% - 121%
AUCTAU (ng•hr/mL)	141.4 (18.4)	140.4 (28.8)	0.97	79% - 121%
CTROUGH (ng/mL)	3.516 (30.0)	3.634 (37.3)	1.01	85% - 120%
CMIN (ng/mL)	3.371 (28.2)	3.501 (354.)	-	-
KEL (hr ⁻¹)	0.050 (17.1)	0.045 (7.8)	-	-
HALF (hr)	14.055 (16.4)	15.567 (7.5)	-	-
CL/F (L/hr)	36 (20.6)	38 (34.4)	-	-
Vd/F (L)	721 (7.8)	842 (26.0)	-	-
TPEAK (hr)	4.667 (24.7)	4.333 (35.3)	-	-
TMIN (hr)	8.000 (173.2)	8.083 (170.5)	-	-

l-Nebivolol

The mean concentration versus time profiles for *l*-neбиволol in EM and PM are depicted in Figure 87.

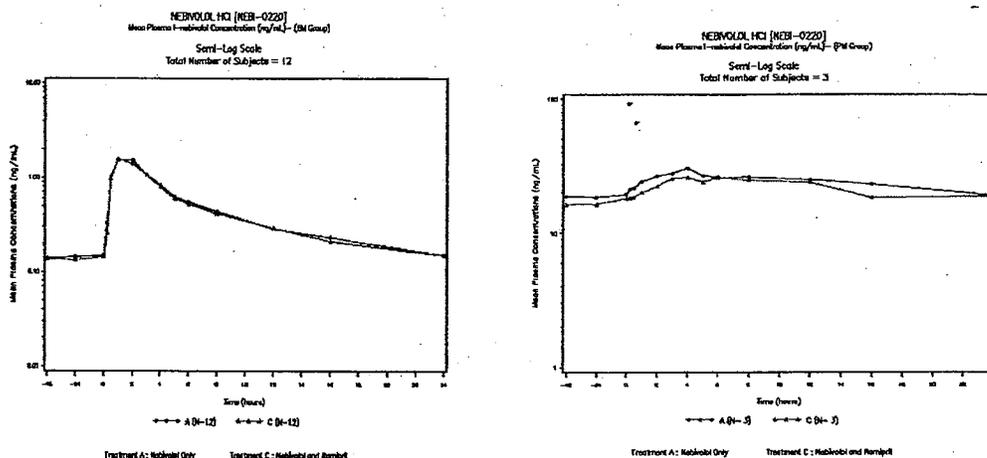


Figure 87: Mean concentration versus time profiles for l-nebivolol in EM and PM
 Pharmacokinetic measures for l-nebivolol in EM subjects are summarized in Table 4.

Based on ANOVA analysis, concomitant administration of ramipril with nebivolol did not produce any statistically significant changes in primary pharmacokinetic measure estimates for l-nebivolol in EM subjects. Only CPEAK was outside the no-effect boundary.

Table 140: Mean (%CV) l-Nebivolol Pharmacokinetic Parameters in Twelve Healthy Male and Female Extensive Metabolizers Following a Daily Oral Dose of 10mg Nebivolol HCL for Ten Days Alone or Concomitantly with a Daily Oral Dose of 5mg Ramipril

Parameter	Treatment A	Treatment C	LSMEANS*	90% Confidence
	Nebivolol	Nebivolol + Ramipril	Ratio (C/A)	Interval**
CPEAK (ng/mL)	1.888 (35.7)	1.797 (27.7)	0.96	79% - 117%
CSS (ng/mL)	0.453 (28.2)	0.435 (22.8)	0.97	91% - 103%
AUCTAU (ng•hr/mL)	10.87 (28.2)	10.43 (22.8)	0.97	91% - 103%
CTROUGH (ng/mL)	0.148 (27.5)	0.143 (26.6)	0.97	90% - 104%
CMIN (ng/mL)	0.139 (26.1)	0.141 (25.3)	-	-
KEL (hr ⁻¹)	0.057 (21.1)	0.060 (11.3)	-	-
HALF (hr)	12.588 (22.5)	11.706 (11.6)	-	-
CL/F (L/hr)	490 (24.1)	502 (22.4)	-	-
Vd/F (L)	8706 (25.7)	8423 (21.2)	-	-
TPEAK (hr)	1.625 (57.4)	1.292 (41.9)	-	-
TMIN (hr)	14.021 (88.0)	10.000 (123.6)	-	-

Pharmacokinetic measures for l-nebivolol in PM subjects are summarized in Table 141.

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Table 141: Mean (%CV) l-Nebivolol Pharmacokinetic Parameters in Three Healthy Male and Female Poor Metabolizers Following a Daily Oral Dose of 10mg Nebivolol HCL for Ten Days Alone or Concomitantly with a Daily Oral Dose of 5mg Ramipril

Parameter	Treatment A Nebivolol	Treatment C Nebivolol + Ramipril	LSMEANS* Ratio (C/A)	90% Confidence Interval**
CPEAK (ng/mL)	30.851 (8.5)	27.251 (6.8)	0.88	76% - 103%
CSS (ng/mL)	24.031 (9.2)	22.060 (10.1)	0.92	89% - 95%
AUCTAU (ng•hr/mL)	576.8 (9.2)	529 (10.1)	0.92	89% - 95%
CTROUGH (ng/mL)	19.352 (13.1)	18.082 (2.1)	0.94	73% - 121%
CMIN (ng/mL)	18.524 (9.1)	16.705 (7.3)	-	-
KEL (hr ⁻¹)	0.024 (20.6)	0.023 (0.9)	-	-
HALF (hr)	29.466 (19.0)	29.804 (0.9)	-	-
CL/F (L/hr)	9 (9.2)	10 (10.5)	-	-
Vd/F (L)	375 (27.4)	385 (3.6)	-	-
TPEAK (hr)	4.667 (24.7)	4.333 (35.3)	-	-
TMIN (hr)	16.000 (86.6)	5.417 (169.2)	-	-

There were no statistically significant changes in l-nebivolol exposure measures in PMs. The 90% CIs were close to the no-effect range for all exposure measures, suggesting that the exposure changes are not likely to be clinically significant. It is noted that the elimination phase (i.e., apparent KEL and HALF) for l-nebivolol could not be adequately characterized in PM subjects, due to relatively flat plasma concentration-time profiles. However, concomitant administration of ramipril with nebivolol did not affect the apparent clearance (CL/F) for l-nebivolol in PM subjects.

Ramipril and Ramiprilat

The plasma concentration-time profile for ramipril is depicted in Figure 88.

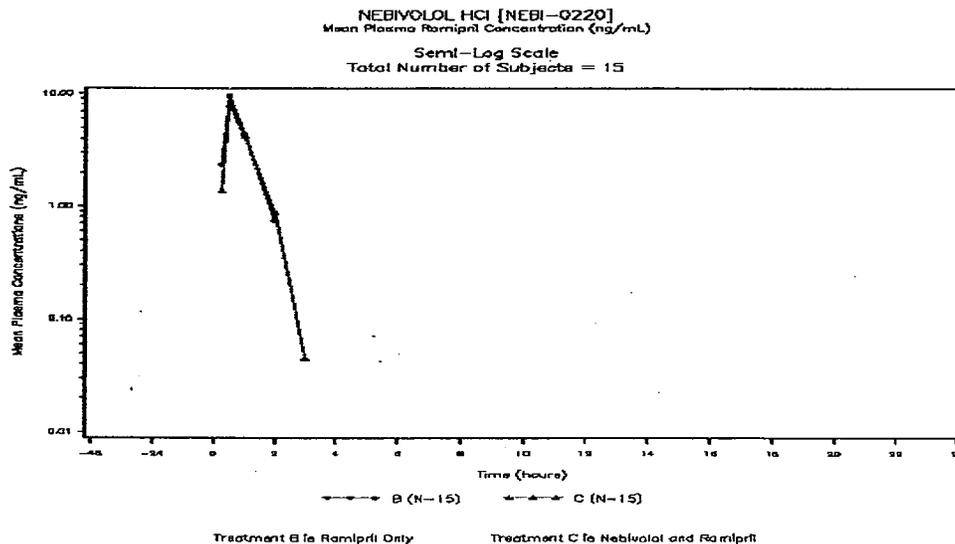


Figure 88: Ramipril Plasma Concentration-Time Profile in presence and absence of Nebivolol

Pharmacokinetic parameters for ramipril (alone or co-administered with nebivolol) could not be accurately estimated due its rapid disappearance from plasma in all 15 subjects. PK measures were estimated from a limited number of samples per subject, and are not considered reliable.

Table 142: Mean (%CV) Ramipril Pharmacokinetic Parameters in Fifteen Healthy Male and Female Subjects Following a Daily Oral Dose of 5mg Ramipril for Ten Days Alone or Concomitantly with a Daily Oral Dose of 10mg Nebivolol HCL

Parameter	Treatment B Ramipril		Treatment C Nebivolol + Ramipril		LS Ratio (AB/B)	90% Confidence Interval for Ramipril
	EM (N=12)	PM (N=3)	EM (N=12)	PM (N=3)	All (N=15)	All (N=15)
CPEAK (ng/mL)	10.987 (53.3)	7.760 (24.9)	9.268 (64.4)	8.567 (61.1)	0.81	59% - 112%
CSS (ng/mL)	0.347 (44.5)	0.309 (19.8)	0.323 (54.3)	0.247 (38.6)	0.87	77% - 99%
AUCTAU (ng·hr/mL)	8.331 (44.5)	7.420 (19.8)	7.760 (54.3)	5.921 (38.6)	0.87	77% - 99%
KEL (hr ⁻¹)	1.548 (38.7)	1.397	1.471 (30.1)		-	-
HALF (hr)	0.517 (47.5)	0.505	0.507 (31.7)		-	-
CL/F (L/hr)	717 (51.3)	691 (18.4)	797 (43.2)	944 (42.1)	-	-
Vd/F(L)	356 (62.8)	557	541 (88.7) b		-	-
TPEAK (hr)	0.583 (33.4)	0.667 (43.3)	0.875 (65.0)	0.667 (43.3)	-	-

Ramiprilat

The plasma concentration time profile for ramiprilat is depicted in Figure 89.

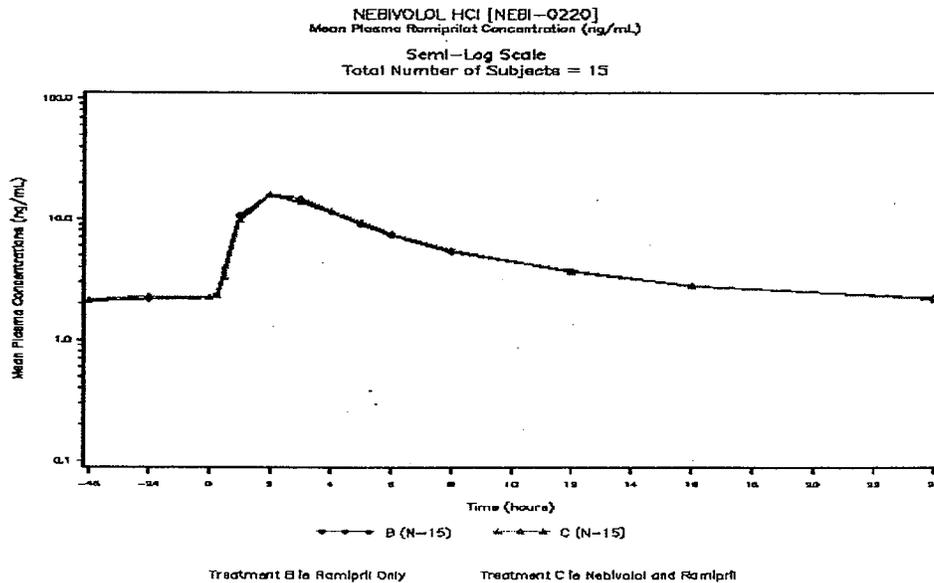


Figure 89: Ramiprilat Plasma Concentration-Time Profile in presence and absence of Nebivolol

Based upon ramiprilat data (CPEAK, CMIN, CSS, AUCTAU, apparent KEL and apparent CL/F; n = 15, Table 143), no treatment differences were observed when nebivolol was co-administered with ramipril (vs. ramipril alone).

Table 143: Mean (%CV) Ramiprilat PK Parameters in 15 Healthy Subjects Following a 5 mg Ramipril QD for Ten Days Alone or with 10mg Nebivolol HCL QD

Parameter	Treatment B Ramipril		Treatment C Nebivolol + Ramipril		LS Ratio* (C/B)	90% CI
	EM (N=12)	PM (N=3)	EM (N=12)	PM (N=3)		
CPEAK (ng/mL)	16.983 (42.6)	11.727 (28.3)	18.006 (45.7)	11.240 (23.9)	1.03	98% - 109%
CSS (ng/mL)	5.524 (26.6)	4.507 (12.7)	5.663 (24.7)	4.384 (17.1)	1.02	99% - 105%
AUCTAU (ng•hr/mL)	132.6 (26.6)	108.2 (12.7)	135.9 (24.7)	105.2 (17.1)	1.02	99% - 105%
CTROUGH (ng/mL)	2.272 (39.2)	2.073 (39.2)	2.287 (29.0)	2.090 (35.9)	1.02	94% - 111%
CMIN (ng/mL)	2.124 (39.2)	1.950 (39.4)	2.045 (25.3)	1.873 (31.3)	-	-
KEL (hr ⁻¹)	0.048 (20.4)	0.049 (10.4)	0.050 (19.6)	0.065 (8.4)	-	-
HALF (hr)	14.9 (19.1)	14.372 (10.9)	14.392 (19.4)	10.720 (8.4)	-	-
CL/F (L/hr)	40 (21.3)	47 (13.5)	38 (19.3)	48 (17.8)	-	-
Vd/F (L)	853 (27.6)	979 (24.9)	784 (22.0)	743 (9.3)	-	-
TPEAK (hr)	1.917 (15.1)	2.333 (24.7)	2.417 (21.3)	2.333 (24.7)	-	-
TMIN (hr)	8.104 (144.9)	8.083 (170.5)	6.125 (176.0)	16.000 (86.6)	-	-

Concomitant administration of ramipril with nebivolol did not produce statistically significant changes in pharmacokinetic parameters estimated for ramipril and ramiprilat, based on ANOVA analysis. Pharmacokinetic parameter estimates for ramiprilat were not affected by co-administration of ramipril with nebivolol.

Applicant's Safety Highlights

There were no serious or life threatening adverse events reported for this study.

Conclusions

There were no drug interactions that would affect the clinical pharmacokinetic profile or the safety of either nebivolol HCL or ramipril upon co-administration.

Labeling Recommendations

The applicant's labeling (below) proposal for the study is acceptable.

Concomitant administration of nebivolol (10 mg once daily) and ramipril (5 mg once daily) for 10 days in 15 healthy adult volunteers produces no pharmacokinetic interactions.

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4.2.26 A Phase I Open-Label Single-Dose Study of the Pharmacokinetic Interaction between Nebivolol HCl and Losartan Potassium in Healthy Volunteers (NEBI-02104)

INVESTIGATORS	James D. Carlson, Pharm.D.
STUDY PERIOD	January 19, 2003 – February 23, 2003

Summary of Drug-Compound interaction Potential for Study Rationale

	Losartan potassium (Cozaar®)	Nebivolol
Typical Use	angiotensin II receptor blocker for treatment of hypertension. Used alone or in combination with other antihypertensive agents.	Proposed for treatment of hypertension
Metabolites (Activity)	EXP-3174 major metabolite (~10 to 40x more potent than losartan, and responsible for most of the activity)	Several metabolites including, glucuronides (major), hydroxy and oxidative metabolites
Metabolic Pathway	substantial first-pass metabolism by cytochrome P450 enzymes. Losartan converted partially to an active carboxylic acid metabolite, EXP-3174. <i>In vitro</i> studies indicate that cytochrome P450 2C9 and 3A4 are involved in the biotransformation of losartan to its metabolites.	CYP2D6 substrate
CYP Inhibitory Potential	None reported	Low potential to inhibit CYP
Interaction Pathway/Mechanism	None expected with nebivolol	None clearly identified.
Highest Recommended Dose/Studied Dose	Initial dose: 50mg QD. Typically administered once or twice daily with total daily doses ranging from 25 mg to 100 mg.	Individualized; initial dose 5 mg QD but expected 10 mg QD

Objective

To determine if co-administration of nebivolol with losartan altered the pharmacokinetics of either nebivolol or losartan.

Study Design

Twenty-four healthy, non-tobacco using, adult, male and female volunteers between the ages of 18 and 55 were accepted into the clinical phase of this study. Subjects were genotyped to determine their CYP2D6 metabolizing status and were randomly assigned to Group 1 or 2 to receive the following treatments:

TREATMENT A: 10mg (1 x 10mg) nebivolol HCL

TREATMENT B: 50mg (1 x 50mg) losartan potassium

TREATMENT C: 10mg (1 x 10mg) nebivolol HCL + 50mg (1 x 50mg) losartan potassium

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Group	Treatment Sequence			Subject	CYP2D6 Metabolic Status
	A	C	B		
1	A	C	B	1, 3, 5, 8, 11, 12, 17, 19, 22, 24	EM
				13, 15	PM
2	B	C	A	2, 4, 6, 7, 9, 10, 18, 20, 21, 23	EM
				14, 16	PM

All doses of nebivolol and/or losartan were given with 240 mL of ambient temperature water. Treatments were given in the fasted state: subjects fasted for at least 10 hours before and until 4 hours after each dosing. Standard meals were provided the evening prior to dosing and at 4 and 10 hours after each dosing.

Subject Characteristics

	All Subjects (n = 24)	Males (n = 14)	Females (n = 10)
Age	30.0 ± 12.2 years	29.7 ± 11.8 years	30.4 ± 13.3 years
Weight	71.0 ± 12.6 kg	78.9 ± 10.0 kg	60.1 ± 5.8 kg
Height	170.0 ± 11.3 cm	177.6 ± 6.9 cm	159.3 ± 6.0 cm

Blood Sampling

Days 1, 15 and Day 29: blood samples were collected prior to dosing and at 0.25, 0.5, 0.75, 1, 1.5, 2, 3, 4, 5, 6, 8, 10, 12, 16, 24, 36, 48, 72, 96, 120 and 144 hours after dosing.

Formulations

- Nebivolol Hydrochloride Tablets (Mylan Pharmaceuticals Inc.), Lot #: R1H1182
- Losartan Potassium Tablets (Merck & Co., Inc.), Lot #: TD402A

Analytical Methods

Losartan Assay

HPLC with tandem mass spectrometric detection was used to determine losartan and EXP-3174 concentrations in human plasma (heparin). The assay performance was acceptable as shown in Table 144.

Table 144: losartan and EXP-3174 Assay Characteristics

Parameter	Measure		Reviewer Comment
Linearity	1.98ng/mL to 792.80ng/mL for losartan	2.04ng/mL to 814.00ng/mL for EXP-3174.	Satisfactory
Between Day Precision (CV %)	5.1% for losartan	≤ 9.5% for EXP-3174	Satisfactory
Between Day Accuracy (%)	For losartan between -3.1% to 3.3%	For EXP-3174, between -1.2% to 2.7%	Satisfactory
LLOQ	1.98ng/mL for losartan	2.04ng/mL for EXP-3174	Satisfactory
Specificity	Sample chromatograms provided that demonstrate assay specificity		Satisfactory

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d- and l-nebivolol assay

HPLC with tandem mass spectrometric detection was used to determine *d*-nebivolol and *l*-nebivolol concentrations in human plasma (heparin). The assay performance was acceptable as shown in Table 145.

Table 145: l- and d-nebivolol Assay Characteristics

Parameter	Measure	Reviewer Comment
	Assay for Extensive metabolizers (Curve III)	
Linearity	0.04ng/mL to 3.0ng/mL	Satisfactory
Precision	d-nebivolol $\leq 8.7\%$	l-nebivolol $\leq 9.0\%$
		Satisfactory
Accuracy	d-nebivolol between -7.0% and 13%	l-nebivolol between 7.6% and 13%
		Satisfactory
LLOQ	0.04ng/mL	Satisfactory
Specificity	Sample chromatograms provided that demonstrate assay specificity	
	Assay for Poor Metabolizers (Curve II)	
Linearity	linear from 0.2ng/mL to 15ng/mL	Satisfactory
between day Precision (CV %)	d-nebivolol $\leq 3.9\%$	l-nebivolol was $\leq 4.3\%$
		Satisfactory
between day Accuracy nominal concentration	d-nebivolol between -6.2% and 6.3%	l-nebivolol between -5.2% and 5.0%
		Satisfactory
LLOQ	0.2ng/mL	Satisfactory
Specificity	Sample chromatograms provided that demonstrate assay specificity	
		Satisfactory

Pharmacokinetics

The following losartan, EXP-3174, *l*-nebivolol and *d*-nebivolol PK measures were estimated: CPEAK, TPEAK, KEL, AUCL, AUCI, THALF, CL/F and Vd/F.

Statistics

Drug-drug interactions were evaluated by standard pharmaco-statistical analyses. The test treatment was nebivolol + losartan and the reference treatment was losartan alone and nebivolol alone.

RESULTS AND DISCUSSION***Subject Disposition***

Twenty-four subjects were enrolled into the study, and twenty-one completed this study. Two subjects withdrew for personal reasons (Subjects 9 and 16) and Subject 20 was dropped by Mylan's PK/DM department prior to Day 29 dose administration due to an upper respiratory tract infection.

Pharmacokinetic and Statistical Analyses***Data Exclusions***

Data are presented for twenty subjects (17 EMs and 3 PMs) in Treatment A (nebivolol alone), twenty-four subjects (20 EMs and 4 EM) in Treatment B (losartan alone), and twenty-three subjects (20 EMs and 3 PMs) in Treatment C (nebivolol and losartan), except where indicated. Data for Subject 12 in Treatment A were excluded from the group analyses due to vomiting that occurred at approximately 2.25 hours (TPEAK = 2

hours for this subject) after nebivolol administration. Moreover, in all PM subjects who were dosed with nebivolol, their pre-dose *l*-nebivolol plasma concentrations in the subsequent dosing period did not return to zero. The *l*-nebivolol plasma concentrations in that period were adjusted to remove the contributions of the leftover *l*-nebivolol concentrations from the preceding period using the subject's apparent elimination rate constant from the previous period.

d-Nebivolol

The mean concentration versus time profiles for *d*-nebivolol in EMs and PMs are illustrated graphically in Figure 90.

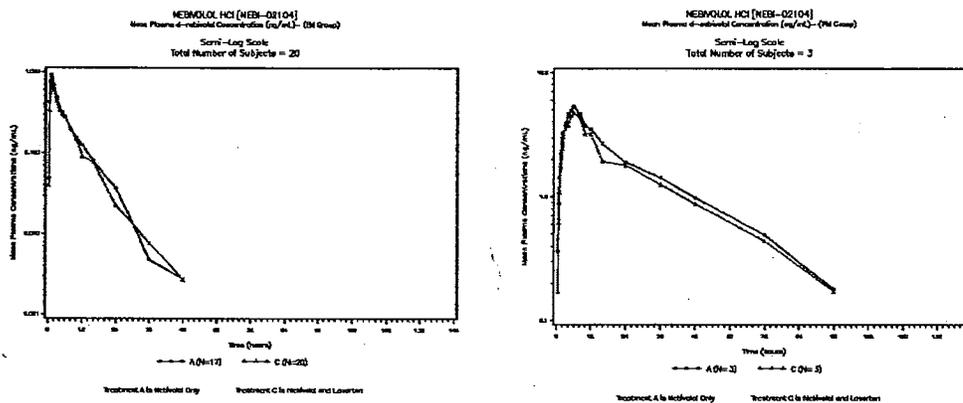


Figure 90: Plasma concentration-time profiles for *d*-nebivolol in the presence and absence of losartan

Pharmacokinetic data for *d*-nebivolol in EMs and PMs are summarized in Table 146 Table 147.

In EMs, concomitant administration of losartan with nebivolol slightly lowered the mean CPEAK of *d*-nebivolol ($p < 0.05$) based on ANOVA analysis, otherwise no statistically significant changes in other exposure measures (i.e. AUCL and AUCI) for *d*-nebivolol were found. The confidence intervals for all exposure measures were outside the no effect range. No statistically significant changes in other pharmacokinetic parameters (TPEAK, HALF, CL/F, Vd/F) for *d*-nebivolol were observed in the presence of losartan.

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Table 146: Mean (%CV) d-Nebivolol PK Parameters in Extensive Metabolizers

Parameter	Arithmetic Mean A = Nebivolol	Arithmetic Mean C = Nebivolol + Losartan	LSMEANS Ratio (C/A)	90% Confidence Interval
AUCL (ng x hr/mL)	4.905 (78.27)	4.264 (88.18)	0.88	77% - 101%
AUCI (ng x hr/mL)	5.573 (70.08)	5.043 (77.95)	0.89	78% - 101%
CPEAK (ng/mL)	1.024 (44.18)	0.808 (42.38)	0.79	68% - 93%
TPEAK (hr)	1.426 (75.83)	1.313 (40.47)		
KEL (hr ⁻¹)	0.087 (18.32)	0.096 (31.87)		
HALF (hr)	8.194 (19.46)	7.886 (30.40)		
CL/F (L/hr)	1169 (47.79)	1387 (54.10)		
Vd/F (L)	13477 (48.52)	14396 (44.21)		

Table 147: Mean (% CV) d-Nebivolol Pharmacokinetic Parameters in Poor Metabolizers Following a Single Oral Dose of 10mg Nebivolol HCL Alone or Concomitantly with a Single Oral Dose of 50mg Losartan Potassium

Parameter	Arithmetic Mean A = Nebivolol	Arithmetic Mean C = Nebivolol + Losartan	LSMEANS Ratio (C/A)*	90% Confidence Interval**
AUCL (ng x hr/mL)	136.0 (19.88)	118.3 (20.92)	0.87	82% - 93%
AUCI (ng x hr/mL)	145.7 (18.01)	127.8 (18.60)	0.88	81% - 95%
CPEAK (ng/mL)	5.448 (19.78)	5.133 (18.65)	0.94	81% - 110%
TPEAK (hr)	5.667 (10.19)	6.333 (24.12)		
KEL (hr ⁻¹)	0.030 (14.41)	0.032 (18.68)		
HALF (hr)	23.41 (15.68)	21.81 (16.99)		
CL/F (L/hr)	35.14 (19.58)	40.05 (18.82)		
Vd/F (L)	1177 (18.22)	1234 (6.565)		

In PMs, concomitant administration of losartan with nebivolol caused a decrease in mean AUCL and AUCI values of *d*-nebivolol ($p < 0.05$), while no significant change in mean CPEAK was observed based on ANOVA analysis. All confidence intervals were within the no effect range. No statistically significant changes were found in other PK measures for *d*-nebivolol (i.e. TPEAK, HALF, Vd/F), except that CL/F was slightly increased by 14% ($p < 0.05$) in the presence of losartan.

l-Nebivolol

The mean concentration versus time profiles for *l*-nebivolol in EMs and PMs are illustrated graphically in Figure 91.

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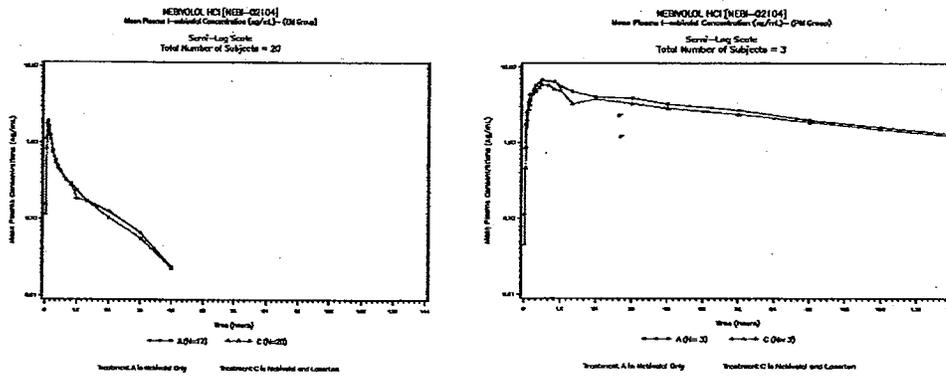


Figure 91: mean concentration versus time profiles for l-nebivolol in EMs and PMs
 Pharmacokinetic data for l-nebivolol in EMs and PMs are summarized in Table 148 and Table 149.

Table 148: Mean (%CV) l-Nebivolol Pharmacokinetic Parameters in Extensive Metabolizers Following a Single Oral Dose of 10mg Nebivolol HCL Alone or Concomitantly with a Single Oral Dose of 50mg Losartan Potassium

Parameter	Arithmetic Mean A = Nebivolol	Arithmetic Mean C = Nebivolol + Losartan	LSMEANS Ratio (C/A)*	90% Confidence Interval**
AUCL (ng x hr/mL)	10.72 (39.74)	9.471 (45.18)	0.88	82% - 95%
AUCI (ng x hr/mL)	11.83 (37.19)	10.64 (41.57)	0.90	84% - 96%
CPEAK (ng/mL)	2.177 (49.00)	1.716 (43.29)	0.80	67% - 94%
TPEAK (hr)	1.294 (79.13)	1.238 (31.76)		
KEL (hr ⁻¹)	0.049 (17.72)	0.049 (18.70)		
HALF (hr)	14.57 (17.21)	14.67 (18.82)		
CL/F (L/hr)	465.9 (28.48)	539.6 (36.69)		
Vd/F (L)	9918 (37.81)	11413 (41.44)		

In EMs, concomitant administration of losartan with nebivolol lowered the mean AUCL, AUCI, and CPEAK values of l-nebivolol ($p < 0.05$) based on ANOVA analysis. The 90% confidence intervals were within the no-effect range for AUC, but the lower boundary of the CI for CPEAK was outside the no-effect range. The clinical significance of decreased CPEAK is unclear. No statistically significant changes were found in other pharmacokinetic measures for nebivolol (i.e. TPEAK, HALF, Vd/F) except that there was an increase in CL/F of 13% ($p < 0.05$) in the presence of losartan.

In PMs, concomitant administration of losartan with nebivolol did not produce any statistically significant changes in the exposure measures for l-nebivolol based on ANOVA analysis.

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Table 149: Mean (%CV) *l*-Nebivolol Pharmacokinetic Parameters in Poor Metabolizers Following a Single Oral Dose of 10mg Nebivolol HCL Alone or Concomitantly with a Single Oral Dose of 50mg Losartan Potassium

Parameter	Arithmetic Mean A = Nebivolol	Arithmetic Mean C = Nebivolol + Losartan	LSMEANS Ratio (C/A)*	90% Confidence Interval**
AUCL (ng x hr/mL)	416.9 (14.87)	365.1 (15.92)	0.88	72% - 107%
AUCI (ng x hr/mL)	567.2 (27.80)	513.7 (13.16)	0.92	66% - 128%
CPEAK (ng/mL)	6.933 (14.52)	6.666 (23.36)	0.95	66% - 137%
TPEAK (hr)	7.000 (37.80)	8.000 (25.00)		
KEL (hr ⁻¹)	0.010 (28.95)	0.012 (73.90)		
HALF (hr)	74.50 (33.38)	75.25 (53.52)		
CL/F (L/hr)	9.249 (25.48)	9.844 (12.90)		
Vd/F (L)	946.0 (15.40)	1035 (51.27)		

The 90% confidence intervals were outside the no-effect range; this may be partially due to the small sample size for the PM group (n = 3). No statistically significant changes were observed in other pharmacokinetic parameters for *l*-nebivolol (i.e. TPEAK, HALF, CL/F, Vd/F) in the presence of losartan.

Losartan and EXP-3174

The mean concentration versus time profiles for losartan and EXP-3174 are illustrated in graphically in Figure 92.

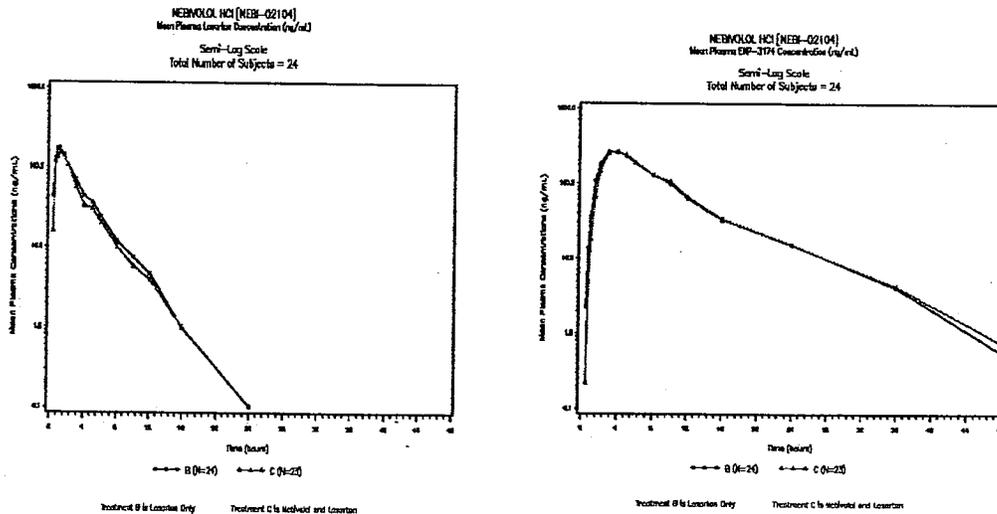


Figure 92: Losartan and EXP-3174 Plasma Concentration-Time Profiles in the presence and absence of nebivolol.

Pharmacokinetic data for losartan and EXP-3174 are summarized in Table 150 and Table 151.

Table 150: Mean (%CV) Losartan Pharmacokinetic Parameters in All Subjects Following a Single Oral Dose of 50mg Losartan Potassium Alone or Concomitantly with a Single Oral Dose of 10mg Nebivolol HCL

Parameter	Arithmetic Mean B Losartan	Arithmetic Mean C = Losartan + Nebivolol	LSMEANS Ratio (C/B)	90% Confidence Interval
AUCL (ng x hr/mL)	532.7 (32.94)	469.9 (42.19)	0.86	81% - 92%
AUCI (ng x hr/mL)	545.4 (32.56)	481.8 (41.71)	0.86	81% - 92%
CPEAK (ng/mL)	266.2 (46.57)	235.2 (52.77)	0.89	77% - 102%
TPEAK (hr)	1.104 (63.31)	0.989 (44.75)		
KEL (hr ⁻¹)	0.298 (24.32)	0.285 (26.81)		
HALF (hr)	2.468 (25.00)	2.603 (26.82)		
CL/F (L/hr)	103.3 (39.59)	122.0 (41.00)		
Vd/F (L)	348.1 (28.24)	425.1 (28.28)		

Concomitant administration of nebivolol with losartan lowered the mean AUCL and AUCI of losartan ($p < 0.05$) based on ANOVA analysis while mean CPEAK was not significantly altered. The 90% confidence intervals were within the no-effect range for AUC, but outside the range for CPEAK. No statistically significant changes were observed in TPEAK and HALF but CL/F and Vd/F were slightly increased by 17% and 21%, respectively ($p < 0.05$), when the drug was co-administered with nebivolol.

Table 151: Mean (%CV) EXP-3174 Pharmacokinetic Parameters in All Subjects Following a Single Oral Dose of 50mg Losartan Potassium Alone or Concomitantly with a Single Oral Dose of 10mg Nebivolol HCL

Parameter	Arithmetic Mean B = Losartan	Arithmetic Mean C = Losartan + Nebivolol	LSMEANS Ratio (C/B)*	90% Confidence Interval**
AUCL (ng x hr/mL)	2302 (24.60)	2245 (26.54)	0.98	94% - 103%
AUCI (ng x hr/mL)	2345 (24.08)	2280 (26.19)	0.98	94% - 102%
CPEAK (ng/mL)	299.6 (33.46)	275.4 (33.86)	0.94	86% - 103%
TPEAK (hr)	3.771 (40.63)	3.826 (21.80)		
KEL (hr ⁻¹)	0.107 (15.52)	0.100 (11.18)		
HALF (hr)	6.642 (15.44)	7.016 (11.29)		
CL/F' (L/hr)	22.49 (23.74)	23.37 (26.03)		
Vd/F' (L)	217.1 (31.81)	235.3 (28.60)		

The presence of nebivolol did not cause any statistically significant changes in the exposure measures for EXP-3174 based on ANOVA analysis. Furthermore, other pharmacokinetic parameters (i.e. TPEAK, HALF, CL/F' and Vd/F') of EXP-3174 were not significantly altered by co-administration of nebivolol with losartan.

Applicant's Safety Analysis

Clinical laboratory, vital sign and ECG monitoring indicated no safety risk associated with oral dosing of 10mg nebivolol HCL tablet alone or concomitantly with 50mg of losartan potassium.

Conclusions

- Nebivolol does not appreciably affect the exposure (PK) of losartan
- Losartan tends to decrease the exposure of nebivolol; particularly the CPEAK by ~ 20 %, and the maximal decrease in AUC was 13 %.

Labeling Recommendations

The label should reflect the study findings. The applicant's labeling language should be modified as follows.

Concomitant administration of nebivolol (10 mg single dose) and losartan (50 mg single dose) in 20 healthy adult volunteers decreased nebivolol C_{max} by approximately 20 % and decreased AUC by approximately 13 %. The change in nebivolol exposure is not considered clinically significant. Nebivolol did not alter losartan pharmacokinetics.

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4.2.27 A Phase I Open-Label Study of the Effect of Repeated-Dose Activated Charcoal on the Pharmacokinetics of Nebivolol HCl in Healthy Volunteers (#: NEBI-02118)

INVESTIGATORS	Thomas S. Clark, M.D., M.S. a
STUDY PERIOD	January 18, 2003 - March 14, 2003

Summary of Nebivolol-Charcoal Interaction Potential (Study Rationale)

	Activate Charcoal (Oral)	Nebivolol
Typical Use	Removal [^] of toxic agents in treatment of acute poisoning	Proposed for treatment of hypertension
Metabolites	NA	Several metabolites including, glucuronides (major), hydroxy and oxidative metabolites
Metabolic Pathway	NA	CYP2D6 substrate
CYP Inhibitory Potential	NA	Low potential to inhibit CYP
Interaction Pathway/Mechanism	Adsorbs drug molecules ultimately enhancing elimination* of some drugs and toxic substances even after systemic absorption by: 1) enhancing transfer rate of drug from the splanchnic circulation back into the gut lumen 2) interrupting the enterohepatic recycling process by adsorbing chemicals excreted into the gastrointestinal tract from bile (Levy, 1982).	None clearly identified.
Highest Recommended Dose/Studied Dose	Typical regimens: 25 g q2 hr or 50 g q4 hr (Martindale, 1996). Given in aqueous suspension without additives or suspending agents. Optimal dosage ratio of activated charcoal to toxin is 10:1 (Actidose-Aqua TM . Package insert)	Individualized, but anticipated to be 10 mg QD

*Activated charcoal (oral administration) increases the clearance of several β -blockers, such as propranolol (al-Meshal, 1993), nadolol (du Souich, 1983), and sotalol (Karkkainen, 1984) in humans or in animals.

[^] Most drugs that are effectively removed by repeated doses of activated charcoal have the following characteristics: undergo enterohepatic or enterenteric circulation, have a small volume of distribution and a low degree of plasma protein binding (MEDLINEplus Health Information).

Note on Charcoal (Safety Considerations)

Activated charcoal is generally non-toxic and well tolerated when given orally, but gastrointestinal disturbances such as vomiting and constipation have been reported.

Study Objective

To determine if nebivolol is subject to enterohepatic recycling by examining the effect of repeated-dose oral activated charcoal on the pharmacokinetics of nebivolol.

Reviewer Note on Study Objective and Study Design

The applicant's objective in conducting the study was to determine if nebivolol undergoes enterohepatic recycling (EHR), rather than to determine if a drug-drug interaction occurs

between charcoal and nebivolol. Consequently, the timing of treatments (charcoal given 4 hours after nebivolol) was not optimal to assess if a drug-drug interaction occurs. This review focuses on the EHR assessment, but recommendations will be made regarding the potential drug-drug interaction.

Study Design

An open-label, randomized, two-period, crossover study design was employed. Fifteen subjects were enrolled in the trial and their CYP2D6 metabolizing status was determined before randomization to Treatments. Each subject received Treatment A and B.

Treatment A

10 mg nebivolol HCL with 240 mL activated charcoal suspension given 4, 8, 12, 16, 22, 28, 36 and 48 hours after nebivolol dosing. Initially, Subjects 1, 4, 5, and 12 received a 50g dose of activated charcoal suspension but the dose was changed to 25 g due to adverse events probably related to charcoal. Subjects 2, 3, 6, 7, 8, 9, 10, 11, 13, 14, and 15 received a 25 mg dose of charcoal suspension.

Treatment B

HCL 10 mg with 240 mL of distilled water at 4, 8, 12, 16, 22, 28, 36 and 48 hours after nebivolol dosing.

Nebivolol

Treatment Sequence		Subject	CYP2D6 Metabolic Status
A	B	1, 4, 5, 6, 13, 15	EM
		9, 12	PM
B	A	2, 3, 7, 8, 14	EM
		10, 11	PM

Fluid and Dietary Requirements

All nebivolol doses were given with 240 mL of ambient temperature water. During the study, subjects were required to consume an additional 180mL of ambient temperature water at 4, 8, 12, 16, 22, 28, 36 and 48 hours after nebivolol dosing (i.e. following each activated charcoal or distilled water administration) to prevent possible charcoal induced constipation. Water was also given at other specified times in the trial. Treatments were given in the fasted state: subjects fasted for at least 10 hours before and until 6 hours after nebivolol dosing. Standard meals were provided during the course of the study.

Subject Characteristics for Subjects who Completed the Study

Sex: 6 males and 3 females
 Age Range (years): 20 – 51
 Weight Range (lb.): 130 – 182

Blood Sample Collection

In each study period, 10mL blood samples were collected prior to nebivolol dosing and at 0.25, 0.5, 1, 2, 3, 4, 5, 6, 7, 8, 10, 12, 14, 16, 22, 28, 36, 48, 72, 96, 120 and 144 hours after nebivolol dosing.

Drugs Studied

- Nebivolol HCL Tablets, 10mg Mylan Pharmaceuticals Inc. Lot # R1H1182
- Actidose-Aqua® (activated charcoal), 50g/240 mL Paddock Laboratories, Inc. Lot # 2446827

Analytical Methods

The concentrations of *d*-neбиволol and *l*-neбиволol in human plasma (heparin) were determined by HPLC with tandem mass spectrometric detection. Assay performance was acceptable as shown in Table 152.

Table 152: Assay Characteristics for d- and l-Nebivolol

Parameter	Measure	Reviewer Comment	
	Assay for Extensive Metabolizers (Curve III)		
Linearity	linear from 0.04ng/mL to 3.0ng/mL	Satisfactory	
CV (%) Between day Precision	d-neбиволol $\leq 3.5\%$	l-neбиволol $\leq 3.4\%$	Satisfactory
Relative Bias Between day Accuracy	d-neбиволol between -5.6% and 5.6%	l-neбиволol between -5.4% and 5.4%	Satisfactory
LLOQ	0.04ng/mL	Satisfactory	
Specificity	Chromatograms provided demonstrated assay specificity	Satisfactory	
	Assay for Poor Metabolizers (Curve II)		
Linearity	linear from 0.2ng/mL to 15ng/mL	Satisfactory	
CV : Between day Precision	d-neбиволol $\leq 7.2\%$	l-neбиволol $\leq 8.4\%$	Satisfactory
Relative Bias Between day Accuracy	d-neбиволol between -5.5% and 6.3%	l-neбиволol between -6.7% and 6.2%	Satisfactory
LLOQ	0.2 ng/mL	Satisfactory	
Specificity	Chromatograms provided demonstrated assay specificity	Satisfactory	

Conjugated plus non-conjugated neбиволol (total neбиволol)

Conjugated plus non-conjugated neбиволol (total neбиволol) concentrations in human plasma (heparin) were determined by high performance liquid chromatography with tandem mass spectrometric detection. Assay performance was acceptable (Table 153)

Table 153: Assay Characteristics for total neбиволol

Parameter	Measure	Reviewer Comment
Linearity	linear from 1.0ng/mL to 800ng/mL	Satisfactory
CV (%) Between day Precision	$\leq 7.3\%$	Satisfactory
Relative Bias Between day Accuracy	Between -2.5% and 3.9%	Satisfactory
LLOQ	1.0 ng/mL	Satisfactory
Specificity	Chromatograms provided demonstrated assay specificity	Satisfactory

Pharmacokinetics

The following single-dose pharmacokinetic measures for *d*-neбиволol, *l*-neбиволol, *d,l*-neбиволol, and neбиволol glucuronides (G-UD) were calculated using non-compartmental techniques:

CPEAK, TPEAK, KEL, AUCL, AUCI, THALF, CL/F, (CL/F' for metabolite), Vd/F. For CL/F calculations, the dose of the individual enantiomers is 5 mg and 10 mg for G-UD.

Statistical Analyses

Drug-drug interactions were evaluated by standard pharmacostatistical procedures. The test treatment was nebivolol + charcoal and the reference treatment was nebivolol alone.

RESULTS AND DISCUSSION

Pharmacokinetic and Statistical Analyses

General Note

Data are presented for seven subjects (5 EMs and 2 PMs) in Treatment A (nebivolol + activated charcoal), and ten subjects (7 EMs and 3 PMs) in Treatment B (nebivolol + distilled water). The data from Subjects 1, 4, 5, 12, and 13 were excluded from the analyses completely. In addition, the data from Subjects 8 and 9 in Treatment A were excluded from the group analyses due to vomiting issues. All PM subjects had pre-dose *l*-nebivolol plasma concentrations prior to Period 2 nebivolol dosing. The *l*-nebivolol plasma concentrations of that period were adjusted to remove the contributions from the leftover *l*-nebivolol concentrations of the preceding period using the subject's apparent elimination rate constant from the previous period (i.e. Period 1).

Statistical analyses were performed on the EM data but not the PM data due to an insufficient number of subjects in the PM group ($n = 3$). EM data were highly variably and treatments were unbalanced in some cases, thus some of the arithmetic means and pharmacokinetic parameters between two treatments did not agree with their respective least squares mean ratios (e.g. arithmetic mean A > arithmetic mean B while least squares mean A/B ratio < 1.0). Conversely, %CV of arithmetic mean estimates for PMs were much lower compared to those for EMs despite the small number of subjects in the PM group.

d-Nebivolol

The mean concentration versus time profiles in EMs and PMs for *d*-nebivolol are illustrated graphically in Figure 93.

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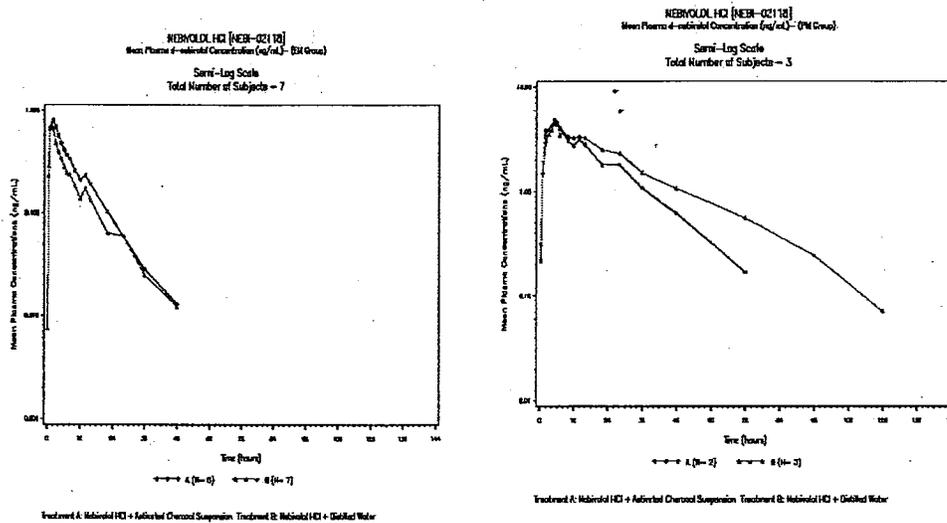


Figure 93: d-nebivolol Plasma Concentration–Time Profiles in Extensive (EMs) and Poor Metabolizers (PMs) with and without coadministration with charcoal (per applicant)

Pharmacokinetic data for *d*-nebivolol in EMs and PMs are presented in Table 154 and Table 155, respectively.

Table 154: d-nebivolol PK measures in the presence and absence of charcoal

Parameter	Arithmetic Mean (CV %)		LSMEANS Ratio (A/B)	90% Confidence Interval
	Treatment A = Nebivolol + Activated Charcoal (n = 5)	Treatment B = Nebivolol + Distilled Water (n = 7)		
AUCL (ng x hr/mL)	7.848 (145.6)	5.913 (132.4)	0.84	55% - 128%
AUCI (ng x hr/mL)	8.645 (132.4)	6.744 (120.1)	0.84	58% - 122%
CPEAK (ng/mL)	0.932 (88.30)	0.802 (53.10)	0.87	51% - 148%
TPEAK (hr)	1.600 (55.90)	1.429 (37.42)	1.13	75% - 150%
KEL (hr ⁻¹)	0.075 (22.59)	0.084 (73.70)	1.31	98% - 164%
HALF (hr)	9.641 (24.63)	11.34 (58.11)	0.68	25% - 112%
CL/F (L/hr)	1244 (55.85)	1481 (77.70)	1.27	80% - 174%
Vd/F ¹ (L)	17605 (63.07)	20101 (69.42)	0.81	38% - 124%

In EMs, repeated-dose activated charcoal did not produce statistically significant changes in *d*-nebivolol pharmacokinetic parameters based on ANOVA analysis. The 90% confidence intervals were outside the 80-125% range, which could be partly due to the low number of subjects involved in the analyses as well as the high within subject variability of nebivolol pharmacokinetics. There were no statistically significant differences between treatments for the other PK measures.

PK Data in PMs were limited and insufficient to make definitive conclusions.

Table 155: d-nebivolol PK measures in the presence and absence of charcoal

Parameter	Arithmetic Mean (CV %)	
	A = Nebivolol+Activated Charcoal (n = 2)	B=Nebivolol+Distilled Water (n =3)
AUCL (ng x hr/mL)	105.7 (23.72)	146.0 (21.87)
AUCI (ng x hr/mL)	114.2 (22.37)	154.5 (21.44)
CPEAK (ng/mL)	4.889 (7.752)	4.739 (10.01)
TPEAK (hr)	5.500 (12.86)	5.000 (20.00)
KEL (hr ⁻¹)	0.046 (24.06)	0.032 (24.26)
HALF (hr)	15.49 (24.06)	22.55 (21.52)
CL/F (L/hr)	44.91 (22.37)	33.49 (23.48)
Vd/F ¹ (L)	976.7 (1.737)	1056 (9.285)

l-Nebivolol

The mean concentration versus time profiles for *l*-nebivolol in EMs and PMs are illustrated graphically in Figure 94.

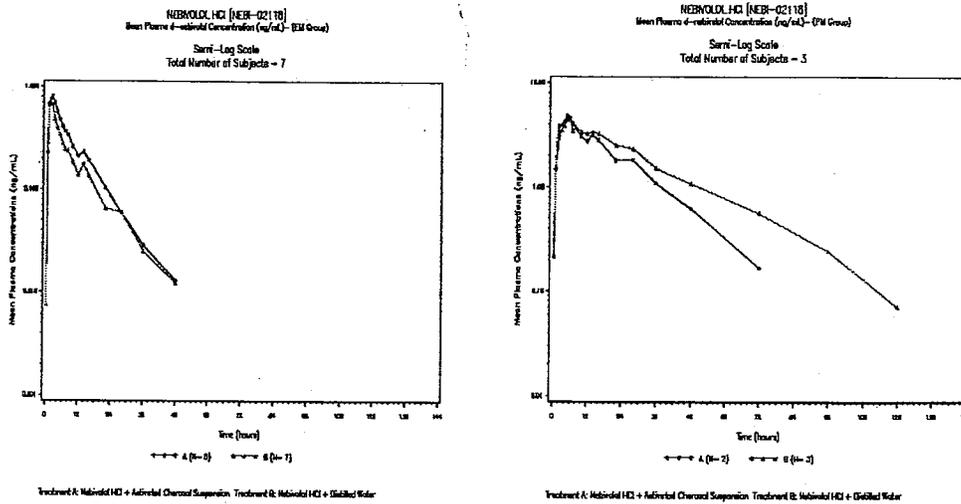


Figure 94: Plasma concentration-time profiles for *l*-nebivolol in EMs and PMs

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Pharmacokinetic data for *l*-nebivolol in EMs and PMs are presented in Table 156 and Table 157, respectively.

Table 156: *l*-nebivolol PK measures in the presence and absence of charcoal in EMs

Parameter	Arithmetic Mean		LSMEANS Ratio (A/B)	90% Confidence Interval
	A = nebivolol+ Activated Charcoal (n = 5)	B = nebivolol+ Distilled Water (n = 7)		
AUCL (ng x hr/mL)	11.08 (90.61)	9.772 (68.27)	0.91	76% - 109%
AUCI (ng x hr/mL)	12.55 (79.66)	11.14 (60.43)	0.94	83% - 107%
CPEAK (ng/mL)	1.486 (55.07)	1.478 (30.09)	0.91	62% - 134%
TPEAK (hr)	1.400 (39.12)	1.214 (46.69)	1.14	71% - 158%
KEL (hr ⁻¹)	0.038 (18.02)	0.042 (22.15)	0.90	76% - 105%
HALF (hr)	18.76 (20.24)	17.26 (21.95)	1.11	97% - 125%
CL/F (L/hr)	539.3 (43.65)	542.6 (36.72)	1.09	100% - 117%
Vd/F ¹ (L)	14995 (59.43)	13963 (54.83)	1.22	105% - 139%

In EMs, repeated-dose activated charcoal did not produce statistically significant changes in *l*-nebivolol PK parameters based on ANOVA analysis. The lower boundary of the 90% confidence interval for LNAUCL and CPEAK was just outside the no effect 80 – 125 range, whereas AUCI was within the no effect range. These findings suggest that there is a lack of effect of activated charcoal on the elimination of *l*-nebivolol. None of the other PK measures were altered significantly during the activated charcoal treatment compared to the distilled water treatment.

In PMs, the PK measures appeared to differ between treatments; however, there was an insufficient number of subjects to make definitive conclusions

Table 157: *l*-nebivolol PK measures in the presence and absence of charcoal in PMs

Parameter	Arithmetic Mean (CV %)	
	A = Nebivolol+Activated Charcoal (n = 2) (n = 2)	B=Nebivolol+Distilled Water (n =3) (n =3)
AUCL (ng x hr/mL)	356.9 (0.339)	483.3 (7.883)
AUCI (ng x hr/mL)	508.6 (6.973)	726.1 (13.81)
CPEAK (ng/mL)	6.136 (0.028)	6.127 (7.275)
TPEAK (hr)	6.000 (23.57)	12.00 (44.10)
KEL (hr ⁻¹)	0.008 (9.719)	0.008 (11.29)
HALF (hr)	89.62 (9.719)	91.52 (11.29)
CL/F (L/hr)	9.854 (6.973)	6.971 (13.23)
Vd/F (L)	1270 (2.754)	917.5 (14.45)

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Nebivolol Glucuronide (G-UD)

The mean concentration versus time profile for G-UD is illustrated graphically in Figure 95.

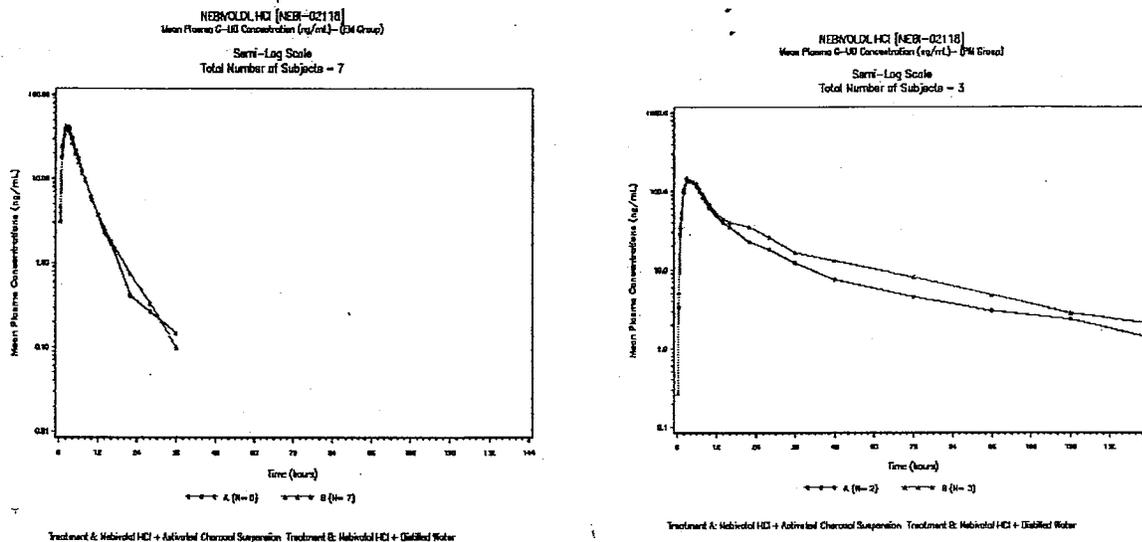


Figure 95: Nebivolol-glucuronide plasma concentration-time profiles in EMs and PMs

Pharmacokinetic data for G-UD in EMs and PMs are presented in Table 158 and Table 159, respectively.

In EMs, G-UD AUC was lower in the activated charcoal treatment relative to the nebivolol alone treatment. However, there were no significant differences in most of the other PK measures, apart from the apparent oral clearance.

Table 158: Nebivolol-GUD PK measures in the presence and absence of charcoal in EMs

Parameter	Arithmetic Mean		LSMEANS Ratio (A/B)	90% Confidence Interval
	A = Nebivolol+ Activated Charcoal (n = 5)	B = Nebivolol+ Distilled Water (n = 7)		
AUCL (ng x hr/mL)	223.8 (50.14)	222.9 (40.46)	0.86	78% - 94%
AUCI (ng x hr/mL)	230.1 (49.67)	231.3 (39.47)	0.85	77% - 94%
CPEAK (ng/mL)	43.17 (36.56)	41.87 (30.21)	0.91	80% - 104%
TPEAK (hr)	2.600 (21.07)	2.143 (17.64)	1.29	96% - 163%
KEL (hr ⁻¹)	0.195 (38.15)	0.143 (39.57)	1.47	97% - 197%
HALF (hr)	4.395 (65.63)	5.664 (43.68)	0.70	35% - 105%
CL/F' (L/hr)	49.59 (31.77)	49.23 (40.39)	1.19	110% - 129%
Vd/F'(L)	268.0 (22.92)	358.9 (27.01)	0.78	43% - 114%
MR ³	15.19 (40.44)	16.68 (37.59)	0.90	77% - 103%

In PMs, the following observations were made (Table 8): 1) both mean AUCL and AUCI decreased by 21% after repeated-dose activated charcoal administration; 2) the mean CL/F' and Vd/F' increased by 27% and 23%, respectively, following activated charcoal administration; and

3) The remaining PK measures did not change. The observed changes appear to be due to a change in bioavailability, rather than CL.

Table 159: Nebivolol-GUD PK measures in the presence and absence of charcoal in PMs

PK Measure	Arithmetic Mean	
	A = Nebivolol+Activated Charcoal (n = 2)	B=Nebivolol+Distilled Water (n = 3)
AUCL (ng x hr/mL)	2069 (22.12)	2603 (20.74)
AUCI (ng x hr/mL)	2147 (22.34)	2723 (18.29)
CPEAK (ng/mL)	137.1 (23.37)	151.3 (21.98)
TPEAK (hr)	3.500 (20.20)	3.667 (31.49)
KEL (hr ⁻¹)	0.018 (13.60)	0.018 (19.09)
HALF (hr)	38.60 (13.60)	38.67 (18.40)
CL/F' (L/hr)	4.777 (22.34)	3.749 (16.55)
Vd/F' (L)	262.0 (8.872)	212.9 (31.96)
MR ^s	3.523 (27.97)	3.421 (25.38)

Discussion

EMs vs. PMs

Overall, the effects of activated charcoal appear to differ slightly between EMs and PMs compared to the drastic differences observed in the metabolic profiles between the two. However data from PMs are insufficient to allow for reliable cross-population comparisons. PMs metabolize mainly by glucuronidation. Activated charcoal appears to have a less striking effect on nebivolol and nebivolol-GUD than it had on other beta blockers. On the basis of this study's results, there is only limited, if any, extent of enterohepatic recycling of nebivolol and G-UD in human. These results are in agreement with earlier findings in rats, in which about one-fifth of the biliary radioactivity was subjected to enterohepatic recycling after an oral dose of nebivolol (Mannens, 1994).

Drug-Drug Interaction

As noted previously, (See *Reviewer Note on Study Objective and Study Design*), the study design was not optimal to determine if a drug-drug interaction occurs between nebivolol and charcoal. The data in PMs suggest that charcoal increases the apparent oral clearance of nebivolol, most likely by decreasing the bioavailability. However, there were an insufficient number of subjects ($n \leq 3$ in the treatment groups) to assure reliability of these clearance observations. Furthermore, the apparent change in clearance may be an additive effect (multiple charcoal doses were given), rather than the clearance associated with a single dose of charcoal that is typically expected. Based on historical data with other beta-blockers it is likely that if charcoal and nebivolol were administered simultaneously, charcoal would have increased nebivolol clearance. Consequently, based on the information from PMs and the historical data, precautionary language regarding the potential increase in nebivolol clearance when coadministered with charcoal should be included in the nebivolol labeling.

Applicant's Safety Analysis

There were no serious or life threatening adverse events reported for this study. According to the applicant, clinical laboratory, vital sign and ECG monitoring indicated no safety risk associated with oral dosing of 10 mg nebivolol HCL tablet alone or concomitantly with 25g/240mL ($\times 8$ doses) activated charcoal suspension. Four subjects (1, 4, 5, and 12) received nebivolol plus

activated charcoal at a dose level of 50g/240mL. However, all four subjects experienced nausea and vomiting after the second or third dose of activated charcoal and were discontinued from the study because of these adverse events. When the activated charcoal dosage was reduced, seven (7) subjects successfully completed all required doses of activated charcoal suspension without experiencing the mentioned adverse events. However, Subject 13 was discontinued from the study prior to Period 2 dosing due to positive β -HCG test.

Conclusions

- There were no striking changes in the pharmacokinetics of nebivolol and G-UD in extensive metabolizers following repeated-dose activated charcoal administration.
- Data were insufficient in poor metabolizers to make definitive conclusions regarding the effect of charcoal nebivolol pharmacokinetics

Recommendation (Labeling)Enterohepatic Recycling

Information from this study was inadequate to determine the extent or significance of enterohepatic recycling on nebivolol pharmacokinetics. The applicant has not proposed including any specific information on this study.

Drug-Drug Interaction Potential (Labeling)

Based on the clearance observations in poor metabolizers (increased nebivolol clearance in the presence of charcoal) and historical data with beta blockers, precautionary labeling language regarding a potential nebivolol-charcoal interaction should be included in the label.

Labeling Language

Concomitant administration of activated charcoal with nebivolol may decrease nebivolol exposure by as much as 20 %.

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4.2.28 An *in vitro* study on protein binding interactions of *rac*-neбиволol with other drugs in human plasma

Report Number: R 67555/FK1038
Dept of Drug Metabolism and Pharmacokinetics, Janssen Research Foundation, Belgium.
Report Completed Date: May 1993.

Objective

To determine if the plasma protein binding of nebivolol is affected by other drugs and vice versa.

Study Description

Standard equilibrium dialysis procedures were used to determine the following:

1) plasma protein binding of 1 ng/mL nebivolol in the presence of the following commonly prescribed drugs (incubated at high therapeutic concentrations): diphenylhydantoin, sulfamethazine, indomethacin, warfarin, propranolol, hydrochlorothiazide, digitoxin (digoxin), and enalapril.

2) The effect of nebivolol (25 ng/mL- supra-therapeutic concentration) on the plasma protein binding of other drugs.

The specific concentrations for the drugs are provided in Tables 1 and 2 under Results. Both radio-labeled and unlabeled drugs were used in the study. Human plasma was obtained from five healthy male volunteers who had not taken any medication for two weeks. The dialysis was carried out against 0.067 M Sorensen phosphate buffer, pH 7.17. The cut-off weight for the dialysis membranes was 12,000 – 14,000 daltons.

Reviewer Comment on Study Procedures and Study Drugs

The study procedures used are acceptable. It should be noted that the degree of plasma protein binding of l- nebivolol (98.13 %) and d-neбиволol (97.85 %) are comparable. Furthermore, the two enantiomers did not undergo a plasma protein binding (displacement) interaction; plasma protein binding was not significantly altered in the presence of the other enantiomer at the same concentration (1 ng/mL). Thus, quantification of *rac*-neбиволol (d- and l-neбиволol) appears acceptable.

According to the applicant, the drugs were selected on the basis of their different binding proteins and binding sites: 1) diazepam, warfarin and digitoxin are marker drugs of the three main drug binding sites on the human serum albumin molecule, 2) imipramine is bound mainly to α_1 acid glycoprotein. The other drugs were selected based on their likelihood of coadministration with nebivolol in the treatment of cardiovascular disease.

Results

The protein binding results for the effect of nebivolol on other drugs and the effect of other drugs on nebivolol are summarized in Tables* Table 160 and Table 161, respectively.

*Reviewer's Note on Interpretation of Results in Tables

The tables provide values for duplicate analyses (mean \pm SD). For statistically significant interactions, the percentage increase in free fraction of *rac*-neбиволol is given in parentheses to aid interpretation of the results because the applicant inadvertently failed to include the values for the controls (percentage bound in absence of co-incubated drug) in the tables.

The mean plasma protein binding of *rac*-neбиволol in this study was $97.74 \pm 0.21\%$ which is comparable to historical data, where *rac*-neбиволol plasma protein binding was 97.50 %.

Table 160: Influence of other drugs on *in vitro* plasma protein binding of *rac*-neбиволol (per Applicant)

Blank plasma ¹ + drug added	% bound <i>rac</i> -neбиволol mean \pm S.D. ²	probability value ³
control	97.82 \pm 0.22	
+ 200 ng/ml imipramine	97.76 \pm 0.22 (+ 2.8 %)	p < 0.01
control	97.82 \pm 0.22	
+ 20 μ g/ml diphenylhydantoin	97.74 \pm 0.22	p > 0.10
control	97.82 \pm 0.22	
+ 3 μ g/ml diazepam	97.72 \pm 0.23 (+ 4.6 %)	p < 0.05
control	97.76 \pm 0.21	
+ 100 μ g/ml tolbutamide	97.55 \pm 0.18 (+ 9.4 %)	p < 0.05
control	97.76 \pm 0.21	
+ 100 μ g/ml sulfamethazine	97.68 \pm 0.27	p > 0.10
control	97.76 \pm 0.21	
+ 3 μ g/ml indomethacin	97.75 \pm 0.19	p > 0.50
control	97.76 \pm 0.24	
+ 10 μ g/ml warfarin	97.79 \pm 0.18	p > 0.10
control	97.76 \pm 0.24	
+ 100 ng/ml propranolol	97.81 \pm 0.20	p > 0.10
control	97.76 \pm 0.24	
+ 500 ng/ml hydrochlorothiazide	97.67 \pm 0.33	p > 0.10
control	97.63 \pm 0.21	
+ 20 ng/ml digitoxin	97.65 \pm 0.21	p > 0.50
control	97.63 \pm 0.21	
+ 100 ng/ml enalapril	97.56 \pm 0.23	p > 0.10

¹ Fortified with ³H-*rac*-neбиволol (1 ng/ml), final ethanol concentration : 1 %.

² n = 5.

³ Determined by two-tailed Student's t-test for paired samples with respect to values obtained for control samples (only ³H-*rac*-neбиволol added) in one run of experiments.

As shown in Table 160, neбиволol free fractions were increased in the presence of imipramine, diazepam and enalapril. However, none of the increases were greater than 10 %

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Table 161: Influence of other drugs on *in vitro* plasma protein binding of *rac*-neбиволol (per Applicant)

Blank plasma*		<i>rac</i> -neбиволol (ng/ml)	% bound drug (mean \pm S.D.) ¹	probability value ²
+ ³ H-diazepam	(0.5 μ g/ml)	0	98.65 \pm 0.12	p > 0.10
+ ³ H-diazepam	(0.5 μ g/ml)	25	98.68 \pm 0.13	
+ ³ H-imipramine	(100 ng/ml)	0	86.46 \pm 0.42	p > 0.50
+ ³ H-imipramine	(100 ng/ml)	25	86.54 \pm 0.81	
+ ³ H-digitoxin	(10 ng/ml)	0	97.05 \pm 0.13	p > 0.05
+ ³ H-digitoxin	(10 ng/ml)	25	97.19 \pm 0.18	
+ ¹⁴ C-warfarin	(5 μ g/ml)	0	99.15 \pm 0.11	p > 0.50
+ ¹⁴ C-warfarin	(5 μ g/ml)	25	99.15 \pm 0.11	
+ ¹⁴ C-diphenylhydantoin	(10 μ g/ml)	0	85.92 \pm 1.06	p > 0.50
+ ¹⁴ C-diphenylhydantoin	(10 μ g/ml)	25	85.84 \pm 0.90	
+ ³ H-propranolol	(40 ng/ml)	0	86.85 \pm 2.29	p > 0.50
+ ³ H-propranolol	(40 ng/ml)	25	86.77 \pm 2.27	
+ ¹⁴ C-hydrochlorothiazide	(100 ng/ml)	0	41.79 \pm 7.50	p > 0.50
+ ¹⁴ C-hydrochlorothiazide	(100 ng/ml)	25	42.57 \pm 2.77	

* final ethanol concentration : 1 %.

¹ N = 5.² Determined by two-tailed Student's t-test for paired samples with respect to values obtained for control samples (no *rac*-neбиволol added) in one run of experiments.

As shown in Table 161, neбиволol did not alter the plasma protein binding of co-incubated drugs.

Discussion

The only significant plasma protein binding interaction occurred between neбиволol and the following drugs: imipramine, diazepam and enalapril. Overall, the increase in neбиволol free fraction was small, < 10 % and is unlikely to be clinically significant. The utility of *in vitro* protein binding displacement interaction information is unclear; because the *in vitro* environment may not be predictive of what will occur *in vivo*. Furthermore, very few protein binding displacement interactions have been observed clinically.

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Conclusions

- Nebivolol is bound approximately 98 % by plasma proteins
- Nebivolol does not cause significant displacement in the plasma protein binding of diazepam, digoxin, diphenylhydantoin, hydrochlorothiazide, imipramine, or warfarin at their therapeutic concentrations
- Nebivolol *in vitro* plasma protein binding is not affected by digoxin, diphenylhydantoin, hydrochlorothiazide, indomethacin, propranolol, sulfamethazine, tolbutamide, or warfarin. Although imipramine, diazepam and enalapril cause statistically significant increases in nebivolol unbound concentrations, the increases are less than 10 % and are not likely to be clinically significant.

Labeling Recommendations

The applicant intends to include the findings from this study in the nebivolol label. The applicant's labeling proposal is acceptable, however, as noted above in the Discussion, the clinical utility of this information is unclear.

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4.3 Filing and Review Form

Office of Clinical Pharmacology and Biopharmaceutics				
New Drug Application Filing and Review Form				
General Information About the Submission				
	Information		Information	
NDA Number	21-742	Brand Name	none	
OCPB Division (I, II, III)	DIV-1	Generic Name	Nebivolol	
Medical Division	CARDIORENAL	Drug Class	Beta-blocker	
OCPB Reviewer	ELENA MISHINA	Indication(s)		
OCPB Team Leader	P. Marroum	Dosage Form	Tablets 2.5, 5, and 10 mg	
		Dosing Regimen	Starting from 5 mg QD up to 40 mg QD	
Date of Submission	April 30, 2004	Route of Administration	oral	
Estimated Due Date of OCPB Review		Sponsor	Bertek Pharmaceuticals	
PDUFA Due Date	February 28, 2005	Priority Classification	S	
Division Due Date				
Clin. Pharm. and Biopharm. Information				
	"X" if included at filing	Number of studies submitted	Number of studies reviewed	Critical Comments If any
STUDY TYPE				
Table of Contents present and sufficient to locate reports, tables, data, etc.	X			
Tabular Listing of All Human Studies	X			
HPK Summary	X			
Labeling	X			
Reference Bioanalytical and Analytical Methods	X			
I. Clinical Pharmacology				
Mass balance:	X	2		
Isozyme characterization:	X	3		
Blood/plasma ratio:	X	1		
Plasma protein binding:	X	1		
Pharmacokinetics (e.g., Phase I) -				
Healthy Volunteers-				
single dose:	X	3		
multiple dose:	X	1		
Patients-				
single dose:				
multiple dose:	X	1		
Dose proportionality -				
fasting / non-fasting single dose:	X	1		
fasting / non-fasting multiple dose:				
Drug-drug interaction studies -				
In-vivo effects on primary drug:	X	7		
In-vivo effects of primary drug:	X	7		
In-vitro:	X	10		
Subpopulation studies -				
ethnicity:				
gender:				
pediatrics:				
geriatrics:				
renal impairment:	X	1		
hepatic impairment:	X	1		
PD:				
Phase 2:				
Phase 3:	X	1		
PK/PD:				
Phase 1 and/or 2, proof of concept:				
Phase 3 clinical trial:				
Population Analyses -	X	1		
Data rich:	X	2		

Data sparse:	X	1		
II. Biopharmaceutics				
Absolute bioavailability:				
Relative bioavailability -				
solution as reference:	X	1		
alternate formulation as reference:	X	1		
Bioequivalence studies -				
traditional design; single / multi dose:	X	1		
replicate design; single / multi dose:				
Food-drug interaction studies:	X	1		
Dissolution:	X			
(IVIVC):				
Bio-wavier request based on BCS	X			
BCS class				
III. Other CPB Studies				
Genotype/phenotype studies:	X			
Chronopharmacokinetics				
Pediatric development plan				
Literature References	X			
Electrophysiology Study	1			
Pharmacodynamic studies	61			
Total Number of Studies Reviewed	22			
Filability and QBR comments				
	"X" if yes	Comments		
Application filable ?	X			
Comments sent to firm ?				
QBR questions (key issues to be considered)				
Other comments or information not included above				
Primary reviewer Signature and Date				
Secondary reviewer Signature and Date				

CC: NDA 21-742, HFD-850(Lee), HFD-860 (Marroum, Mehta, Mishina), Biopharm (CDER)

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/s/

Elena Mishina
1/28/05 04:43:51 PM
BIOPHARMACEUTICS

Robert Kumi
1/31/05 09:35:07 AM
BIOPHARMACEUTICS
Patrick, My section is okay... Note: overdose section (applicant
reported) already has some information about charcoal.

Patrick Marroum
1/31/05 03:32:07 PM
BIOPHARMACEUTICS