Table 29: Mean (%CV) l-Nebivolol PK Parameters

PROTOCOL NUMBER NEBI-0127							
EM GROUP N=12	Treatment A 10mg tablet - fasting	Treatment B 10mg tablet - fed	Treatment C 10mg solution -fasting	Least Square Mean Ratio*(%)		90% Confidence Interval*(%)	
				B/A	A/C	B/A	A/C
AUCL (ng x hr/mL)	11.76 (54.99)	11.10 (56.00)	13.07 (53.76)	94.4	88.9	84.9-105	79.9-98.9
AUCI (ng x hr/mL)	12.56 (52.64)	12.11 (51.97)	13.89 (51.57)	97.0	89.4	87.9-107	81.0-98.7
CPEAK (ng/mL)	1.831 (32.57)	1.495 (49.09)	2.248 (41.76)	75.1	83.8	62.8-89.8	70.0-100
TPEAK (hr)	1.375 (51.72)	2.333 (55.83)	0.958 (41.37)				
KEL (hr^{-1})	0.042 (15.80)	0.045 (19.29)	0.041 (18.00)				
HALF (hr)	16.74 (15.36)	16.12 (19.95)	17.44 (17.36)				
CL/F[¥] (L/hr)	493.9 (45.49)	507.5 (45.56)	435.1 (41.35)				
Vd/F[¥] (L)	11580 (39.55)	11245 (35.39)	10629 (35.77)				
PM GROUP N=6[§]	Treatment A 10mg tablet - fasting	Treatment B 10mg tablet - fed	Treatment C 10mg solution -fasting	Least Square Mean Ratio*(%)		90% Confidence Interval*(%)	
				B/A	A/C	B/A	A/C
AUCL (ng x hr/mL)	262.0 (18.84)	299.7 (24.86)	255.6 (14.53)	113	102	108-119	97.1 -107
AUCI (ng x hr/mL)	579.9 (33.32)	764.9 (68.91)	464.3 (22.32)	119	120	95.7-149	94.4 -153
CPEAK (ng/mL)	5.544 (23.94)	6.167 (15.02)	4.948 (14.99)	113	110	100 -127	97.8 - 124
TPEAK (hr)	4.333 (27.95)	4.167 (46.58)	5.333 (82.73)				
KEL (hr^{-1})	0.009 (30.33)	0.009 (37.45)	0.013 (20.49)				
HALF (hr)	79.51 (30.58)	87.84 (49.71)	56.65 (23.79)				
CL/F[¥] (L/hr)	9.195 (22.76)	8.210 (37.55)	11.26 (24.96)				
Vd/F[¥] (L)	1009 (25.11)	887.7 (19.52)	888.5 (14.06)				

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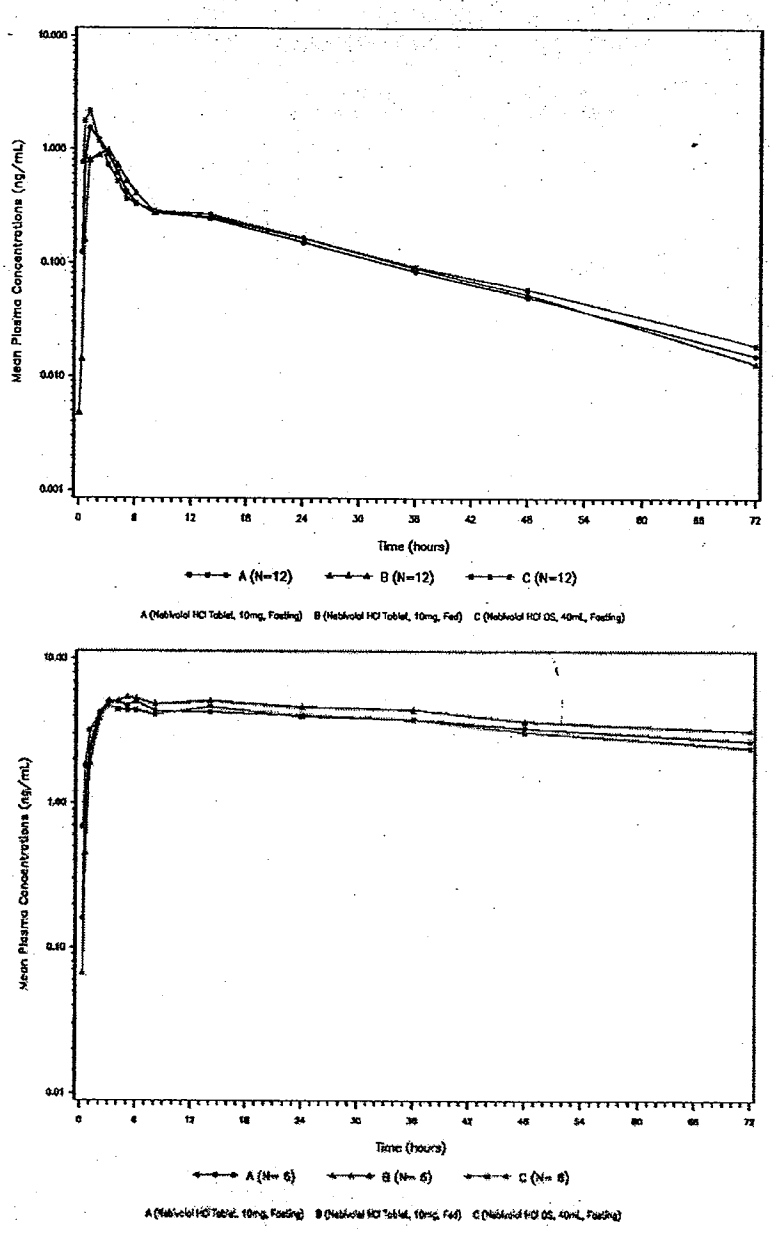

Figure 18: Mean Plasma l-Nebivolol Concentrations vs. Time in EM (upper panel) and PM (lower panel) subjects

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Table 30: Mean (%CV) d,l-Nebivolol PK Parameters

PROTOCOL NUMBER NEBI-0127							
EM GROUP N=12	Treatment A 10mg tablet - fasting	Treatment B 10mg tablet - fed	Treatment C 10mg solution -fasting	Least Square Mean Ratio*(%)		90% Confidence Interval*(%)	
				B/A	A/C	B/A	A/C
AUCL (ng x hr/mL)	18.59 (65.17)	19.79 (56.93)	21.17 (67.14)	109	86.9	96.6-124	76.7-98.4
AUCI (ng x hr/mL)	19.30 (64.17)	20.76 (54.61)	21.97 (65.69)	111	86.8	98.3-125	77.0-97.9
CPEAK (ng/mL)	2.978 (38.29)	2.709 (52.33)	3.618 (53.51)	83.3	85.4	68.8-101	70.6-103
TPEAK (hr)	1.375 (51.72)	2.333 (55.83)	1.000 (36.93)				
KEL (hr^{-1})	0.052 (24.94)	0.060 (19.23)	0.049 (20.22)				
HALF (hr)	14.29 (28.07)	11.99 (18.20)	14.64 (21.88)				
CL/F[†] (L/hr)	686.6 (47.53)	612.8 (48.78)	586.1 (43.53)				
Vd/F[†] (L)	13721 (48.50)	10003 (37.13)	12077 (41.50)				
PM GROUP N=6[§]	Treatment A 10mg tablet - fasting	Treatment B 10mg tablet - fed	Treatment C 10mg solution -fasting	Least Square Mean Ratio*(%)		90% Confidence Interval*(%)	
				B/A	A/C	B/A	A/C
AUCL (ng x hr/mL)	377.5 (19.67)	442.8 (24.47)	370.3 (17.26)	116	102	112-121	97.9-105
AUCI (ng x hr/mL)	631.0 (24.75)	762.8 (40.31)	563.3 (18.43)	117	111	104-132	98.9-125
CPEAK (ng/mL)	9.753 (26.81)	11.26 (17.87)	8.527 (19.48)	117	113	100-137	96.6-132
TPEAK (hr)	4.167 (41.34)	4.000 (44.72)	5.333 (82.73)				
KEL (hr^{-1})	0.013 (19.62)	0.014 (27.96)	0.016 (18.95)				
HALF (hr)	53.32 (17.83)	54.26 (30.20)	45.12 (18.84)				
CL/F[†] (L/hr)	16.50 (19.59)	14.45 (28.55)	18.33 (20.57)				
Vd/F[†] (L)	1252 (21.50)	1073 (26.09)	1171 (17.62)				

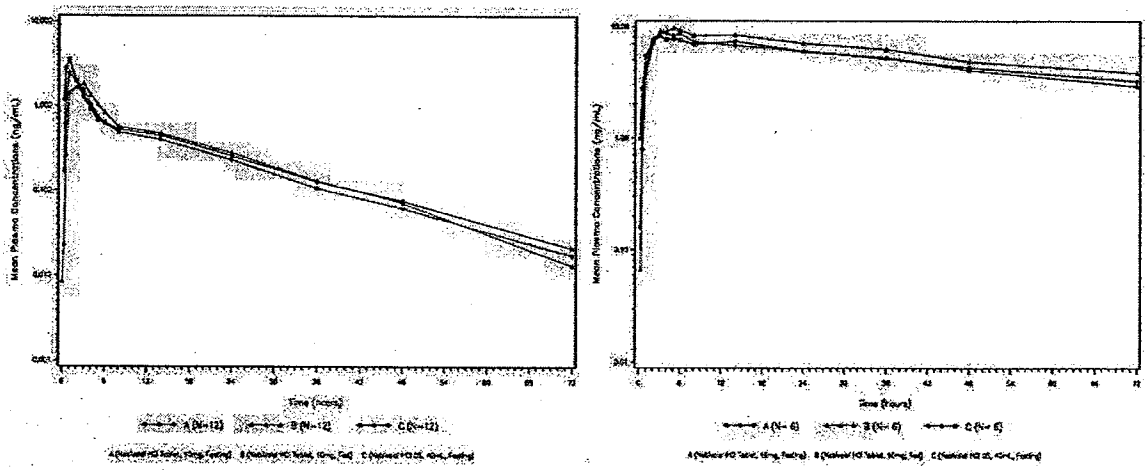

Figure 19: Mean Plasma d,l-Nebivolol Concentrations vs. Time in EM (left panel) and PM (right panel) subjects

There was no statistically significant difference between the pharmacokinetic parameters of nebivolol glucuronides in EMs when nebivolol was administered under fasting conditions as either a tablet or a liquid. Nebivolol glucuronides did not exhibit a food effect in PM subjects (least square mean ratios are 109%, 116%, and 86.5%, respectively for LnAUCL, LnAUCI, and LnCPEAK). For the EMs, the LnAUCL, LnAUCI, and LnCPEAK values of nebivolol glucuronides under fed conditions were about 70% of the same values estimated under fasting conditions (Table 5).

Table 31: Mean (%CV) Nebivolol Glucuronides PK Parameters

PROTOCOL NUMBER NEBI-0127							
EM GROUP N=12	Treatment A 10mg tablet - fasting	Treatment B 10mg tablet - fed	Treatment C 10mg solution -fasting	Least Square Mean Ratio*(%)		90% Confidence Interval*(%)	
				B/A	A/C	B/A	A/C
AUCL (ng x hr/mL)	281.8 (45.02)	218.3 (71.46)	286.2 (41.06)	71.5	98.0	64.6 – 79.2	88.5 – 108
AUCI (ng x hr/mL)	283.9 (44.97)	219.9 (71.08)	287.5 (41.08)	71.6	98.3	64.7 – 79.2	88.8 – 109
CPEAK (ng/mL)	44.79 (32.99)	31.79 (56.19)	47.60 (31.09)	66.5	93.5	57.6 – 76.7	81.1 – 108
TPEAK (hr)	2.417 (27.66)	3.333 (36.93)	1.833 (39.15)				
KEL (hr^{-1})	0.0898 (45.03)	0.0933 (34.69)	0.0917 (29.12)				
HALF (hr)	9.447 (52.55)	8.184 (30.26)	8.080 (25.69)				
CL/F [$] (L/hr)	39.81 (31.08)	58.28 (40.38)	39.22 (33.95)				
Vd/F [$] (L)	542.2 (63.31)	624.3 (27.89)	445.2 (34.60)				
PM GROUP N=6[§]	Treatment A 10mg tablet - fasting	Treatment B 10mg tablet - fed	Treatment C 10mg solution -fasting	Least Square Mean Ratio*(%)		90% Confidence Interval*(%)	
				B/A	A/C	B/A	A/C
AUCL (ng x hr/mL)	1814 (22.42)	1951 (16.55)	1891 (12.79)	109	94.3	96.8 – 122	83.9 – 106
AUCI (ng x hr/mL)	2066 (23.30)	2389 (21.35)	2243 (20.06)	116	91.3	104 – 130	81.4 – 102
CPEAK (ng/mL)	115.3 (18.95)	98.84 (10.92)	120.0 (15.76)	86.5	95.7	75.8 – 98.8	83.9 – 109
TPEAK (hr)	3.667 (14.08)	4.500 (30.63)	3.500 (15.65)				
KEL (hr^{-1})	0.0276 (14.50)	0.0241 (19.97)	0.0253 (31.22)				
HALF (hr)	25.60 (17.12)	29.74 (18.73)	29.99 (36.29)				
CL/F [$] (L/hr)	5.117 (28.02)	4.385 (25.89)	4.611 (20.12)				
Vd/F [$] (L)	187.0 (27.70)	181.5 (10.57)	189.5 (18.48)				

*Used Log Transformed Parameters

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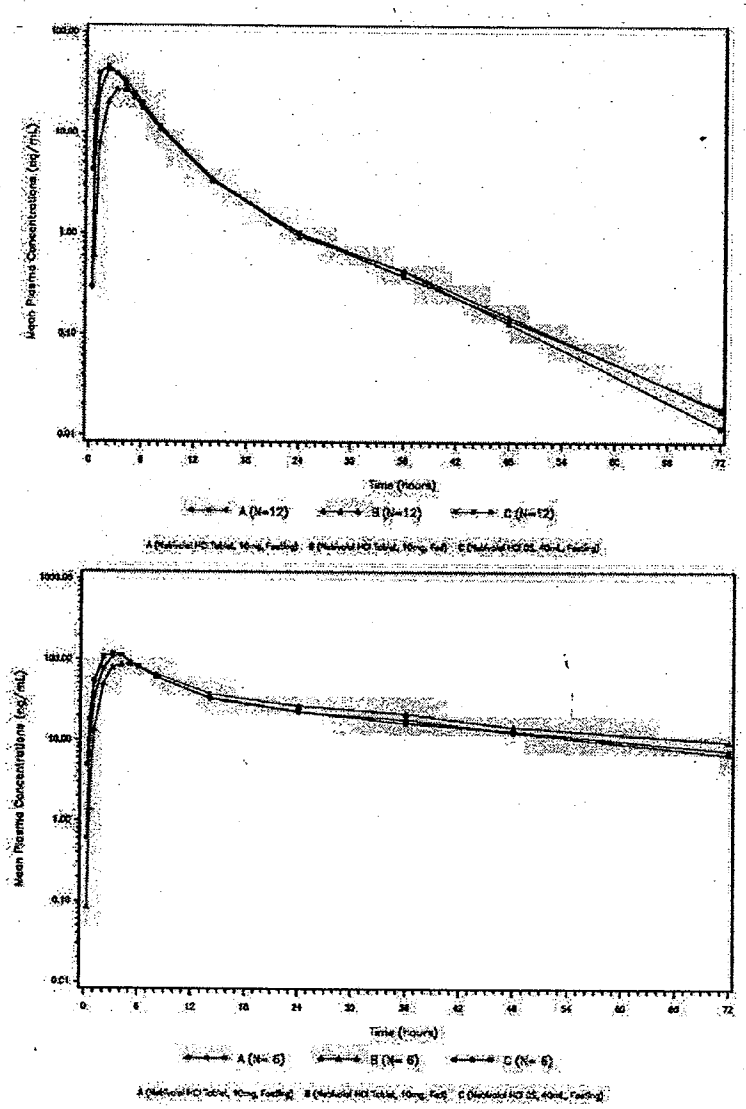

Figure 20: Mean Plasma Nebivolol Glucuronide Concentrations vs. Time in EM (upper panel) and PM (lower panel) subjects

Since nebivolol is administered as a racemic mixture (d, l-nebivolol), which does not exhibit a food effect, the sponsor proposed to administer nebivolol irrespective of meals for all subjects.

COMMENTS:

1. The relative bioavailability (utilizing AUCI) for the nebivolol tablets compared to the oral solution (reference) both administered under fasting conditions was approximately 87% for EMs and roughly 111% for PMs calculated for the combined plasma concentrations of d- and l-nebivolol.

2. The least square mean ratios for d,l-nebivolol (racemic mixture) were all between 80% – 125% for both EM and PM subjects, thus indicating that nebivolol can be administered irrespective of meals for all subjects.
3. Under fed conditions, AUCL and AUCI for nebivolol glucuronides are reduced approximately 30%, and CPEAK levels are diminished roughly 33% in EM subjects as compared to the fasting condition values. The nebivolol glucuronides moiety in PM subjects, however, does not exhibit a food effect.
4. The sponsor should include recommendations 2 and 3 in the PI.

4.2.3 A Phase I Open-Label Single-Dose Study Assessing the Pharmacokinetics of Nebivolol HCL and the Formation of Metabolites in Healthy Volunteers (NEBI-0223)

DRUG STUDIED:	**Nebivolol HCL Tablets, 10mg Mylan Pharmaceuticals Inc. Lot # R1H1182 Manufacture Date: April 17, 2000**
INVESTIGATORS:	**Thomas S. Clark, M. D., M. S.**
STUDY SITE M. D.	
ANALYTICAL SITE:	**Mylan Pharmaceuticals Inc. Bioanalytical Department 3711 Collins Ferry Road Morgantown, WV 26505**
DATE OF STUDY:	**Clinical Phase: April 26, 2002 - April 30, 2002**
	Analytical Phase: June 5, 2002 - June 10, 2002

OBJECTIVES:

To determine the pharmacokinetic profile of nebivolol HCL after a single dose administration.
To detect the formation of any metabolites of nebivolol (d-nebivolol, l-nebivolol, and d,l-nebivolol) in the plasma and urine.

STUDY DESIGN:

Subjects were genotyped to determine their CYP2D6 metabolizing status and were randomized according to their status, either as an extensive metabolizer (EM) or a poor metabolizer (PM). Pharmacokinetic parameters were derived from plasma d-nebivolol, l-nebivolol, and d, l-nebivolol concentration time curves. Eight (4 EM and 4 PM) healthy, non- smoking, male and female subjects between the ages of 22 and 38 completed this open- label, one period study.
Subjects were housed from the evening prior to dosing until 24 hours after dosing. After a supervised overnight fast (at least 10 hours) each subject received a single, oral 10mg tablet with 240mL of ambient temperature water.
For EM subjects, serial blood samples were collected within 30 minutes prior to dosing and at the following times relative to dosing (6 x 10mL): 0.5, 1.0, 1.5, 2.0, 4.0, 8.0, and 24 hours. For PM subjects, serial blood samples were collected within 30 minutes prior to dosing and at the following times relative to dosing (6 x 10mL): 1.0, 2.0, 4.0, 8.0, 14, 24, and 48 hours. All urine from each subject was collected from study check- in until 72 hours after dosing at the following collection intervals: 16- 0, 0- 4, 4- 8, 8- 12, 12- 16, 16- 24, 24- 48, and 48- 72 hours.

ASSAY:

The assay utilized two different standard curve ranges, one for EMs and another for PMs. The HPLC with tandem mass spectrometric detection was used for the analysis of d- and l-nebivolol in human plasma (Table 32). Chromatograms were shown.

Table 32: Assay Characteristics for d- and l-Nebivolol

Parameter	Measure		Reviewer Comment
	Assay for Extensive Metabolizers (Curve III)		
Linearity	0.04ng/mL to 3.0ng/mL		Satisfactory
Precision (CV %)	d-nebivolol ≤ 5.2	l-nebivolol ≤ 4.8	Satisfactory
Accuracy Between day	d-nebivolol between –4.6% and 5.9%	l-nebivolol between -3.3% and 6.8%	Satisfactory
LLOQ	0.04ng/mL		Satisfactory
Specificity			Satisfactory
	Assay for Poor Metabolizers (Curve II)		
Linearity	linear from 0.2ng/mL to 15ng/mL		Satisfactory
Precision (CV %)	d-nebivolol ≤ 5.2	l-nebivolol ≤ 4.8	Satisfactory
Accuracy Between day	d-nebivolol between –6.2 and 7.6%	l-nebivolol between –5.6 and 6.3%	Satisfactory
LLOQ	0.2ng/mL		Satisfactory
Specificity			Satisfactory

Although it was intended, the assay for non-conjugated plus conjugated nebivolol as well as for any of the metabolites was not performed.

RESULTS:

The demographic data are shown in Table 33.

Table 33: Demographic Data

Subject Number	CYP2D6 Genomics Status (Alleles)	Age	Sex	Race	Height (in)	Frame Size	Entry Weight (lbs)	Exit Weight (lbs)
1	EM (*1/*1)	38	M	W	74.00	L	182.00	181.00
2	EM (*1/*4)	24	M	W	70.00	L	192.00	194.00
3	EM (*1/*1)	24	M	W	67.25	L	152.00	152.00
4	EM (*1/*4)	34	F	W	60.50	L	156.00	155.00
5	PM (*4/*5)	37	F	W	64.25	L	144.00	144.00
6	PM (*3/*4)	32	M	W	69.50	L	192.00	194.00
7	PM (*3/*4)	22	M	W	72.00	L	192.00	190.00
8	PM (*4/*5)	25	M	W	69.50	M	164.00	165.00

Individual plasma concentrations of d- and l-nebivolol were added together for each subject to provide an estimate of the d, l-nebivolol plasma concentration. Pharmacokinetic parameters for d-, l- and d, l-nebivolol were calculated using noncompartmental techniques. Statistical analyses were not performed on the pharmacokinetic parameters for d-, l- and d,l-nebivolol. No statistical comparisons were made between the EM and PM subjects. Summary data for the EM group was shown with and without subject 4 whose data do not place her in the EM group.

The urine samples were not assayed.

Table 34-Table 36 provide the summary PK parameters for d-nebivolol, l-nebivolol and d, l-nebivolol in EM and PM subjects.

Table 34: Mean (CV) d-Nebivolol PK parameters

Treatment Group	Parameter							
	AUCL (ng x hr/mL)	AUCI (ng x hr/mL)	CPEAK (ng/mL)	TPEAK (hr)	KEL (hr^{-1})	HALF (hr)	CL/F$^{\$}$ (L/hr)	Vd/F$^{\$}$ (L)
EM (n = 4)	12.56 (155.4)	3.440$^{\#}$ (62.25)	1.105 (96.24)	2.750 (127.3)	0.1694$^{\$}$ (101.1)	7.640$^{\#}$ (75.00)	1805 (118.4)	15708$^{\#}$ (39.40)
EM (n =3)*	2.836 (68.70)	3.440 (62.25)	0.583 (42.33)	1.000 (0.000)	0.1694 (101.1)	7.640 (75.00)	2367 (94.07)	15708 (39.40)
PM (n = 4)	95.94 (42.03)	136.8 (45.54)	3.821 (40.97)	5.500 (54.55)	0.0295 (42.01)	26.00 (30.92)	46.94 (67.79)	1513 (29.86)

* Subject 4 removed from analysis because PK profile was not consistent with EM metabolic status (n = 3 for this parameter

Table 35: Mean (CV) l-Nebivolol PK parameters

Treatment Group	Parameter							
	AUCL (ng x hr/mL)	AUCI (ng x hr/mL)	CPEAK (ng/mL)	TPEAK (hr)	KEL (hr^{-1})	HALF (hr)	CL/F$^{\$}$ (L/hr)	Vd/F$^{\$}$ (L)
EM (n = 4)	14.95 (119.6)	11.09$^{\#}$ (-)	1.588 (42.25)	2.750 (127.3)	0.0624$^{\#}$ (-)	11.12$^{\#}$ (-)	704.9 (74.52)	7227$^{\#}$ (-)
EM (n = 3)*	6.082 (33.42)	11.09$^{\#}$ (-)	1.303 (33.42)	1.000 (0.000)	0.0624$^{\#}$ (-)	11.12$^{\#}$ (-)	899.8 (47.94)	7227$^{\#}$ (-)
PM (n = 4)	176.2 (26.53)	997.6 (46.70)	5.209 (28.66)	8.500 (48.51)	0.0050 (52.79)	175.2 (55.33)	6.098 (55.15)	1260.7 (21.67)

* Subject 4 removed from analysis because PK profile was not consistent with EM metabolic status

Table 36: Mean (CV) d,l-Nebivolol PK parameters

Treatment Group	Parameter							
	AUCL (ng x hr/mL)	AUCI (ng x hr/mL)	CPEAK (ng/mL)	TPEAK (hr)	KEL (hr^{-1})	HALF (hr)	CL/F$^{\$}$ (L/hr)	Vd/F$^{\$}$ (L)
EM (n = 4)	27.56 (135.5)	16.04$^{\#}$ (-)	2.692 (63.34)	2.750 (127.3)	0.0708$^{\$}$ (-)	9.800$^{\#}$ (-)	1006 (84.40)	8806$^{\#}$ (-)
EM (n =3)*	8.982 (50.06)	16.04$^{\#}$ (-)	1.886 (36.01)	1.000 (0.000)	0.0708$^{\#}$ (-)	9.800$^{\#}$ (-)	1302 (57.40)	8806$^{\#}$ (-)
PM (n = 4)	272.2 (31.57)	688.0^ (35.17)	8.922 (34.56)	7.000 (28.57)	0.0122^ (1.159)	56.77^ (1.445)	15.49^ (35.17)	1268^ (34.08)

* Subject 4 removed from analysis because PK profile was not consistent with EM metabolic status

Figure 21, Figure 22, and Figure 23 display the plasma concentrations profiles for d-nebivolol, l-nebivolol and d, l-nebivolol in EM and PM subjects for each treatment.

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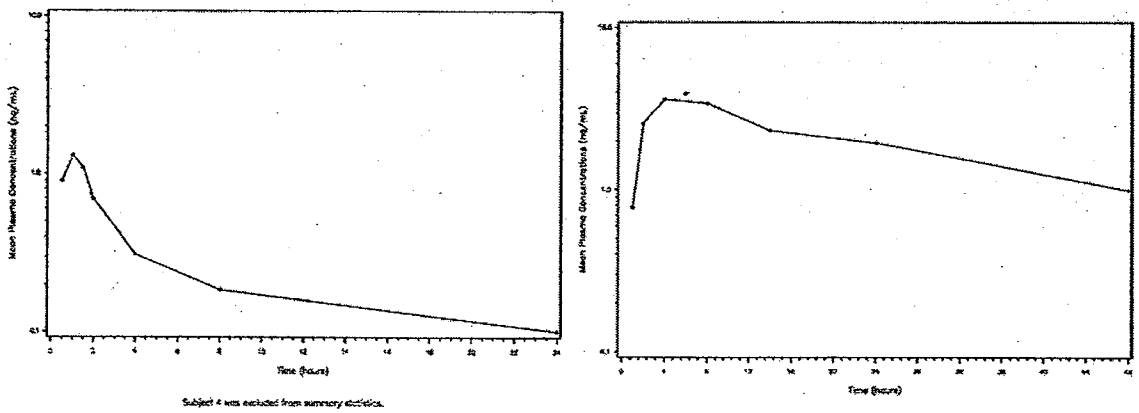

Figure 21: Mean Plasma concentrations of d-nebivolol vs. time. Left panel, EM, right panel, PM

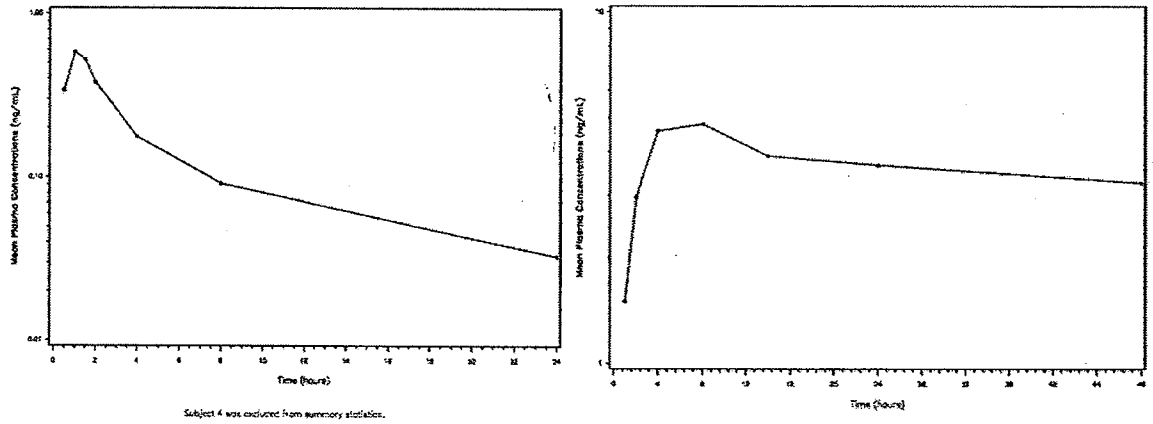

Figure 22: Mean Plasma concentrations of l-nebivolol vs. time. Left panel, EM, right panel, PM

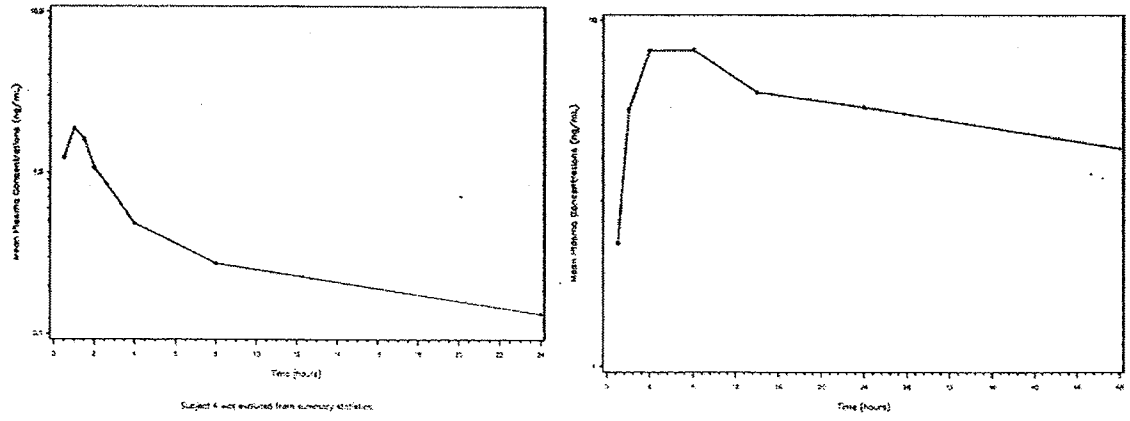

Figure 23: Mean plasma concentrations of d,l-nebivolol vs. time. Left panel, EM, right panel, PM

In three EM subjects (excluding Subject 4), the maximum plasma concentration of d, l-nebivolol was 1.9 ng/mL with a TPEAK around 1 hour after oral dosing. The plasma half-life of d, l-nebivolol in the EM subjects was 9.8 hours. Mean AUCL and AUCI values were 9 and 16 ng·hr/mL, respectively. For d, l-nebivolol plasma concentrations in PM subjects were maximal at 7 hours after oral dosing with a CPEAK of 8.9ng/mL. The half-life is considerably longer in PM subjects (57 hours), resulting in AUCL and AUCI values of 272 and 688 ng·hr/mL, respectively. The apparent oral clearance of d, l-nebivolol in three EM subjects was ten fold larger than that observed for PM subjects (1.3 vs. 15.5 L/hr). The apparent volume of distribution for d, l-nebivolol is larger in EM subjects compared to PM subjects (8.8 vs. 1.3 L).
The sponsor concluded that each enantiomer contributes differently to the overall PK profile seen with d, l-nebivolol in EM and PM subjects.
In EM subjects, d-nebivolol reached CPEAK of 0.6 ng/mL at 1 hour, the same as that observed for d,l-nebivolol. The half-life was 8 hours and the mean AUCL and AUCI values were 2.8 and 3.4 ng·hr/mL, respectively. In PM subjects, d-nebivolol plasma concentrations reached a maximum of 3.8 ng/mL at 5.5 hours after the dose. The half-life for d-nebivolol was longer in PM subjects (26 hours) and AUCL and AUCI values were 96 and 137 ng·hr/mL, respectively. In EM subjects, d-nebivolol was cleared much faster than in PM subjects (apparent oral clearance of 2 vs. 47 L/hr). The apparent volume of distribution for the d-isomer was 10-fold larger in EM subjects compared to PM subjects (15.7 vs. 1.5 L).
In EM subjects, CPEAK of l-nebivolol was 1.3 ng/mL with a TPEAK of 1 hour. The half-life of l-nebivolol in the plasma of EM subjects was 11 hours. Mean AUCL and AUCI values were 6 and 11 ng·hr/mL, respectively. In PM subjects CPEAK of 5.2 ng/mL was reached at 8.5 hours and the half-life was estimated as 175 hours and AUCL and AUCI values were 176 and 998 ng·hr/mL for the EM and the PM groups. The apparent oral clearance of l-nebivolol in three EM subjects was much greater than that observed for PM subjects (900 vs. 6 L/hr) and the apparent volume of distribution for the l-enantiomer is much larger in EM subjects compared to PM subjects (7 vs. 1.3 L).

Sponsor's Conclusions:
Due to impaired metabolism of the drug by CYP2D6, PM subjects have higher plasma concentrations of nebivolol than the EM subjects. The decreased metabolic capacity of the PM subjects resulted in later TPEAK values and longer half- lives. The apparent oral clearance and apparent volume of distribution is smaller in PM subjects than their EM counterparts.

COMMENTS:

1. This was a descriptive study confirming previous finding of the sponsor that there are differences in maximum concentrations of d- and l-nebivolol between EM and PM subjects. The sponsor properly concluded that d-nebivolol and l-nebivolol both have different plasma profiles in subjects which are deficient in the CYP2D6 enzyme.
2. The d- and l- isomers of nebivolol have quite different pharmacokinetic and pharmacologic properties. The sponsor added the plasma concentrations at each time point for both isomers and reported the pharmacokinetic parameters for the so called d,l-nebivolol (non-existed molecular entity). These data presentation is not acceptable since these parameters do not have any physiologic meaning.

3. The sponsor has planned to detect the formation of any metabolites in the studied subjects, however, none of the metabolites measurements was reported. Additionally, urine samples were collected from all subjects but were not assayed by the sponsor.
4. Despite collection of the plasma concentrations data up to 24 hours post-dose in EMs and 48 hours in PMs, the half-life in PMs was calculated as 26 hours for d- and 175 hours for l-nebivolol. These calculations do not deem accurate since the data did not cover at least 3-half-lives measurements needed to obtain good estimation.
5. The calculated pharmacokinetic parameters should be interpreted with caution.

4.2.4 A Phase I Open Label Multiple Dose Study Assessing the Pharmacokinetics of Nebivolol HCL and the Formation of Metabolites in Healthy Volunteers (NEBI-0270)

DRUG STUDIED: **Nebivolol HCL Tablets, 10mg Mylan Pharmaceuticals Inc. Lot # R1H1182 Manufacture Date: April 17, 2000**

INVESTIGATORS: **Thomas S. Clark, M. D., M. S.**

STUDY SITE:

OBJECTIVES:

Primary:

To determine the pharmacokinetic profile of both d- and l-nebivolol and its pharmacologically active metabolites (if possible) in plasma following multiple dose administration.

Secondary:

To compare (for both d- and l-nebivolol) the area under the curve from 0 to infinity (AUCI) following a single- dose of nebivolol to the area under the curve from 0 to the dosing interval of 24- hours (AUCTAU) following obtainment of steady-state conditions.

In addition, urine was collected for investigation into the pharmacological activity of the metabolites of nebivolol.

STUDY DESIGN:

This was an open-label, one-period, multiple-dose study investigating the pharmacokinetics of nebivolol HCL. Twenty adult, tobacco-free, healthy volunteers, consisting of 16 EM and 4 PM subjects, received a single nebivolol HCL tablet (1 x 10 mg) on Day 1 followed by a daily 10 mg (1 x 10 mg) dose of nebivolol for fourteen consecutive days beginning on Day 4. Subjects were housed from 16 hours prior to dosing until 24 hours after dosing on Days 1 and 17. Subjects are to return to the clinic for dosing on the other days and for blood collections scheduled after the 24-hour post-dosing time point. On Days 1 and 17, serial blood samples, 10mL (1 x 10mL) were collected at pre-dose (within 10 minutes prior to dosing), 0.25, 0.5, 1, 2, 3, 4, 6, 8, 10, 12, 16, 24, 36, 48 and 72 hours post- dose. Additional 10mL samples were collected within 10 minutes prior to dosing on Day 15 and Day 16 for determination of steady-state attainment. Urine samples were collected prior to dosing on Day 1 and then on a daily basis until 72 hours after dosing on Day 17.

ASSAY:

The assay utilized two different standard curve ranges, one for EMs and another for PMs. The method for the analysis of d-nebivolol and l-nebivolol in human plasma (heparin) was performed using high performance liquid chromatography with tandem mass spectrometric detection.

Table 37: Assay Characteristics for d- and l-Nebivolol

Parameter	Measure		Reviewer Comment
	Assay for Extensive Metabolizers (Curve III)		
Linearity	0.04ng/mL to 3.0ng/mL		Satisfactory
Precision (CV %)	d-nebivolol $\leq$ 7.6	l-nebivolol $\leq$ 6.2	Satisfactory
Accuracy	d-nebivolol	l-nebivolol	Satisfactory

Between day	between -3.1% and 4.4%	between -3.6% and 6.0%	
LLOQ	0.04ng/mL		Satisfactory
Specificity			Satisfactory
	Assay for Poor Metabolizers (Curve II)		
Linearity	linear from 0.2ng/mL to 15ng/mL		Satisfactory
Precision (CV %)	d-nebivolol $\leq$ 7.9	l-nebivolol $\leq$ 8.3	Satisfactory
Accuracy Between day	d-nebivolol between -8.6 and 3.5%	l-nebivolol between -7.3 and 5.1%	Satisfactory
LLOQ	0.2ng/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.

Table 38: Assay for non-conjugated plus conjugated nebivolol

Parameter	Measure	Comment
Linearity	linear from 1.0ng/mL to 800ng/mL	Satisfactory
Precision (CV %)	$\leq$ 6.2	Satisfactory
Accuracy Between day	between -1.7 and 5.7%	Satisfactory
LLOQ	1.0ng/mL	Satisfactory
Specificity		Satisfactory

Chromatograms were shown.

PHARMACOKINETICS:

Individual plasma concentrations of d-nebivolol and l-nebivolol were added together for each subject to provide an estimate of the d,l-nebivolol plasma concentration. The individual plasma concentrations for d- and l-nebivolol were subtracted from the corresponding conjugated plus non-conjugated nebivolol plasma concentrations to arrive at the individual nebivolol glucuronides plasma concentrations. The parameters were calculated using the noncompartmental method. Statistical analyses were then performed on the individual plasma concentrations for d-nebivolol, l-nebivolol and d,l-nebivolol and nebivolol glucuronides.
To report the magnitude of fluctuation of the concentration- time profile as a percentage of either CSS or CMIN for the steady state, two fluctuation indices were determined.

FLUCT1 = [(CPEAK- CMIN)/CSS] x 100.
FLUCT2 = [(CPEAK- CMIN)/CMIN] X 100.

RESULTS:

The demographic data are shown in Table 39. Twenty- five (25) healthy adult, tobacco- free volunteers were enrolled in this study. Twenty- two subjects (16 EM and 6 PM) between the ages of 19 and 53 completed this study.

Table 39: Demographic Data

Volunteer #	Volunteer ID	Age (Yrs)	Gender	CYP2D6 (alleles)	Height (in)	Frame Size^	Race	Body Weight (lbs) (Day -1)
1	[redacted]7646	22	M	EM (*1/*4)	73.00	L	Caucasian	179.00
2	[redacted]1295	20	M	EM (*1/*1)	67.25	L	Caucasian	181.00
3	[redacted]4440	52	M	EM (*1/*1)	69.00	L	Caucasian	171.00
4	[redacted]2301	31	M	EM (*1/*5)	69.50	M	Caucasian	142.00
5	[redacted]2759	32	M	EM (*1/*5)	64.00	M	Caucasian	132.00
6	[redacted]5113	26	M	PM (*4/*4)	66.00	L	Caucasian	142.00
7	[redacted]3330	19	M	EM (*1/*4)	67.00	L	Caucasian	176.00
8	[redacted]7487	22	M	EM (*1/*1)	72.00	L	Caucasian	202.00
9	[redacted]8341	26	M	EM (*1/*1)	68.00	M	Caucasian	160.00
10	[redacted]6471	38	M	EM (*1/*1)	74.00	L	Caucasian	173.00
11	[redacted]5636	24	M	EM (*1/*5)	67.50	M	Caucasian	177.00
12	[redacted]7303	23	M	EM (*1/*4)	74.00	L	Caucasian	212.00
13	[redacted]1643	53	M	EM (*1/*1)	70.00	M	Caucasian	156.00
14	[redacted]1745	24	M	EM (*1/*4)	70.00	L	Caucasian	190.00
15	[redacted]8737	48	F	PM (*4/*4)	67.00	L	Caucasian	165.00
16	[redacted]7599	26	F	PM (*4/*3)	61.50	L	Caucasian	141.00
17	[redacted]3773	40	F	EM (*1/*1)	64.00	L	Caucasian	142.00
18	[redacted]7758	24	M	PM (*4/*4)	82.00	L	Caucasian	234.00
19	[redacted]8670	23	M	EM (*1/*3)	72.50	L	Caucasian	191.00
20	[redacted]1022	23	M	EM (*1/*1)	74.50	L	Caucasian	184.00
21	[redacted]0328	24	F	EM (*1/*1)	64.00	M	Caucasian	147.00
22	[redacted]7495	29	M	PM (*4/*4)	72.50	L	Caucasian	179.00
23	[redacted]8215	19	F	PM (*4/*5)	63.00	S	Caucasian	120.00
24	[redacted]4465	21	M	PM (*4/*4)	68.00	M	Caucasian	180.00
25	[redacted]6099	30	F	PM (*7/*5)	64.25	M	Caucasian	136.00

An analysis of variance test to assess steady-state attainment was performed using CTROUGH concentration data from the Day 15 - 17 pre-dose plasma samples. The differences between the PK parameters of d-, l-, d,l-nebivolol, and nebivolol glucuronides on Day 1 and Day 17 were assessed statistically. Tables below (Table 40-Table 47) provide the summary PK parameters for d-nebivolol, l-nebivolol and d,l-nebivolol and nebivolol glucuronide in EM and PM subjects after a single and multiple doses of nebivolol.

Table 40: Mean (%CV) d-nebivolol PK parameters (single dose, Day 1)

Parameter	Extensive Metabolizers (n=17)*	Poor Metabolizers (n=7)
AUCL (ng x hr/mL)	6.044 (107.9)	110.6 (16.88)
AUCI (ng x hr/mL)	7.324 (94.45)	122.6 (17.18)
CPEAK (ng/mL)	1.135 (47.90)	4.278 (18.94)
KEL (hr^{-1})	0.060 (26.67)	0.033 (9.395)
HALF (hr)	12.53 (34.08)	20.96 (9.534)
TPEAK (hr)	1.265 (39.83)	3.857 (40.80)
CI/F (L/hr)	922.1 (38.09)	42.01 (20.17)
Vd/F (L)	16154 (42.26)	1269 (21.36)

Table 41: Mean (%CV) d-nebivolol PK parameters (multiple doses, Day 14)

Parameter	Extensive Metabolizers (EMs) (n=16)	EMs Least Squares Mean Ratio (%)[a]	EMs 90% Confidence Interval (%)[a]	Poor Metabolizers (PMs) (n=6)	PMs Least Squares Mean Ratio (%)[b]	PMs 90% Confidence Interval (%)[b]
AUCTAU(ngxhr/mL)	7.513 (109.1)	98.9	87.8 – 112	104.9 (18.23)	87.5	80.4 – 95.2
CPEAK (ng/mL)	1.204 (46.86)	101	90.0 – 114	6.538 (14.81)	155	131 – 183
KEL (hr^{-1})	0.057 (29.31)	101	89.5 – 112	0.033 (25.90)	99.5	83.7 – 115
HALF (hr)	12.92 (25.89)	96.4	84.7 – 108	22.33 (24.82)	105	87.3 – 123
TPEAK (hr)	1.250 (46.19)	102	84.1 – 121	3.833 (30.50)	95.7	61.5 – 130
Cl/F (L/hr)	962.8 (39.87)	103	89.7 – 116	48.96 (17.76)	114	106 – 123
Vd/F (L)	17837 (51.03)	99.1	84.3 – 114	1563 (25.17)	120	96.4 – 143
CTROUGH[c] (ng/mL)	0.112 (116.9)	---	---	2.351 (28.86)	---	---
CSS (ng/mL)	0.313 (109.1)	---	---	4.372 (18.23)	---	---
FLUCT1 (%)	456.8 (38.79)	---	---	99.02 (20.68)	---	---
FLUCT2 (%)	1470 (60.00)	---	---	196.3 (27.65)	---	---

Table 42: Mean (%CV) l-Nebivolol PK parameters (single dose, Day 1)

Parameter	Extensive Metabolizers (n=17)	Poor Metabolizers (n=7)
AUCL (ng x hr/mL)	10.60 (67.77)	268.6 (23.32)
AUCI (ng x hr/mL)	12.09 (62.38)	493.6 (26.68)
CPEAK (ng/mL)	2.076 (41.82)	5.672 (25.63)
KEL (hr^{-1})	0.044 (18.21)	0.012 (9.999)
HALF (hr)	16.34 (18.94)	58.93 (9.624)
TPEAK (hr)	1.176 (42.34)	4.286 (29.25)
Cl/F (L/hr)	490.2 (31.19)	10.89 (30.99)
Vd/F (L)	11264 (33.86)	914.3 (26.80)

Table 43: Mean (%CV) l-Nebivolol PK parameters (multiple doses, Day 14)

Parameter	Extensive Metabolizers (EMs) (n=16)	EMs Least Squares Mean Ratio (%)[a]	EMs 90% Confidence Interval (%)[a]	Poor Metabolizers (PMs) (n=6)	PMs Least Squares Mean Ratio (%)[a]	PMs 90% Confidence Interval (%)[a]
AUCTAU(ngxhr/mL)	12.22 (67.73)	97.3	91.9 – 103	528.3 (21.44)	114	105 – 125
CPEAK (ng/mL)	2.251 (37.58)	104	91.4 – 118	25.83 (23.51)	475	423 – 534
KEL (hr^{-1})	0.044 (20.84)	101	92.2 – 110	0.011 (40.41)	89.3	63.3 – 115
HALF (hr)	16.69 (26.45)	101	90.6 – 111	73.37 (38.32)	126	90.1 – 162
TPEAK (hr)	1.250 (46.19)	111	84.7 – 137	5.667 (89.75)	131	44.7 – 218
Cl/F (L/hr)	503.6 (36.49)	104	98.0 – 110	9.834 (21.25)	85.4	73.8 – 97.0
Vd/F (L)	12274 (52.52)	109	93.7 – 125	1038 (41.10)	110	70.8 – 149
CTROUGH[c] (ng/mL)	0.176 (76.65)	---	---	16.82 (28.48)	---	---
CSS (ng/mL)	0.509 (67.73)	---	---	22.01 (21.44)	---	---
FLUCT1 (%)	458.5 (31.86)	---	---	43.40 (31.32)	---	---
FLUCT2 (%)	1503 (47.13)	---	---	59.42 (34.97)	---	---

Table 44: Mean (%CV) d,l-Nebivolol PK parameters (Single Dose, Day 1)

Parameter	Extensive Metabolizers (n=17)	Poor Metabolizers (n=7)
AUCL (ng x hr/mL)	17.00 (81.66)	379.2 (21.08)
AUCI (ng x hr/mL)	18.10 (77.77)	572.0 (23.46)
CPEAK (ng/mL)	3.190 (41.80)	9.852 (22.09)
KEL (hr^{-1})	0.061 (16.43)	0.016 (10.14)
HALF (hr)	11.62 (16.33)	43.93 (9.79)
TPEAK (hr)	1.176 (42.34)	4.000 (38.19)
CI/F (L/hr)	690.4 (33.11)	18.51 (27.78)
Vd/F (L)	11364 (35.43)	1161 (23.96)

Table 45: Mean (%CV) d,l-Nebivolol PK parameters (multiple doses, Day 14)

Parameter	Extensive Metabolizers (EMs) (n=16)	EMs Least Squares Mean Ratio (%)*	EMs 90% Confidence Interval (%)*	Poor Metabolizers (PMs) (n=6)	PMs Least Squares Mean Ratio (%)*	PMs 90% Confidence Interval (%)*
AUCTAU(ngxhr/mL)	19.73 (83.14)	105	98.0 – 113	633.2 (19.34)	117	108 – 126
CPEAK (ng/mL)	3.453 (39.53)	103	91.3 – 117	32.11 (19.79)	336	302 – 373
KEL (hr^{-1})	0.057 (17.87)	92.9	85.5 – 100	0.013 (28.09)	81.7	66.2 – 97.2
HALF (hr)	12.66 (20.67)	109	99.1 – 118	56.05 (25.23)	128	106 – 150
TPEAK (hr)	1.188 (33.95)	105	85.1 – 126	3.667 (14.08)	91.7	51.2 – 132
CI/F (L/hr)	657.4 (37.32)	96.1	88.9 – 103	16.32 (20.05)	84.1	74.2 – 94.0
Vd/F (L)	11859 (45.62)	105	88.9 – 121	1314 (29.25)	110	86.5 – 133
CTROUGH[3] (ng/mL)	0.288 (92.07)	---	---	19.17 (25.26)	---	---
CSS (ng/mL)	0.822 (83.14)	---	---	26.38 (19.34)	---	---
FLUCT1 (%)	455.4 (34.25)	---	---	51.61 (27.42)	---	---
FLUCT2 (%)	1598 (57.39)	---	---	74.07 (31.47)	---	---

Figures below show the plasma concentrations vs. time profiles for d- and l-nebivolol.

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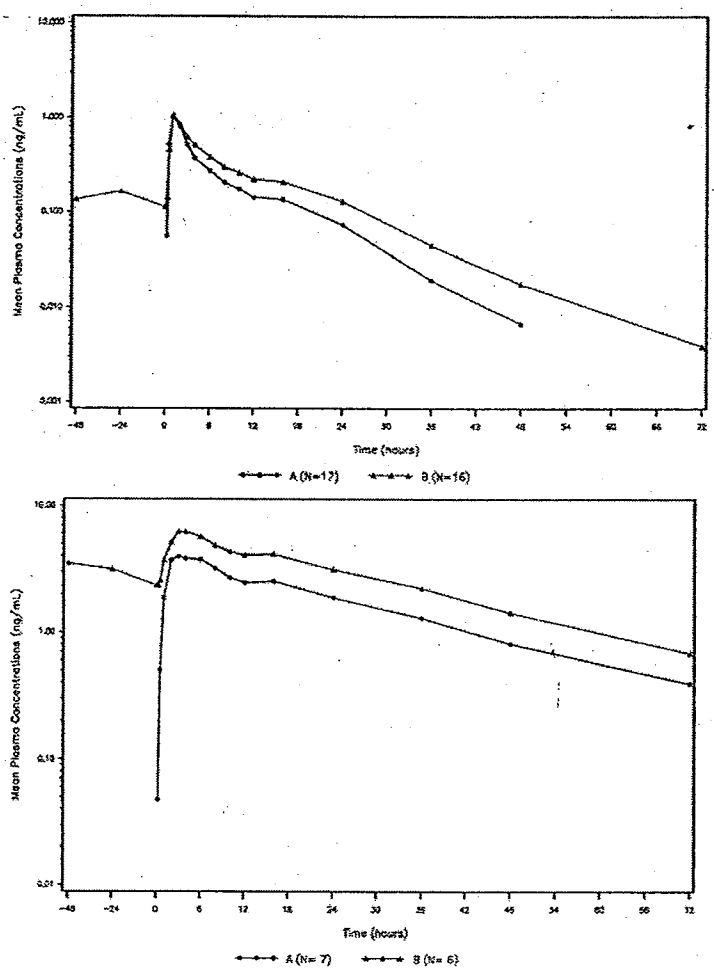

Figure 24: Mean plasma concentrations of d-nebivolol vs. time. Upper panel, EM, low panel, PM.

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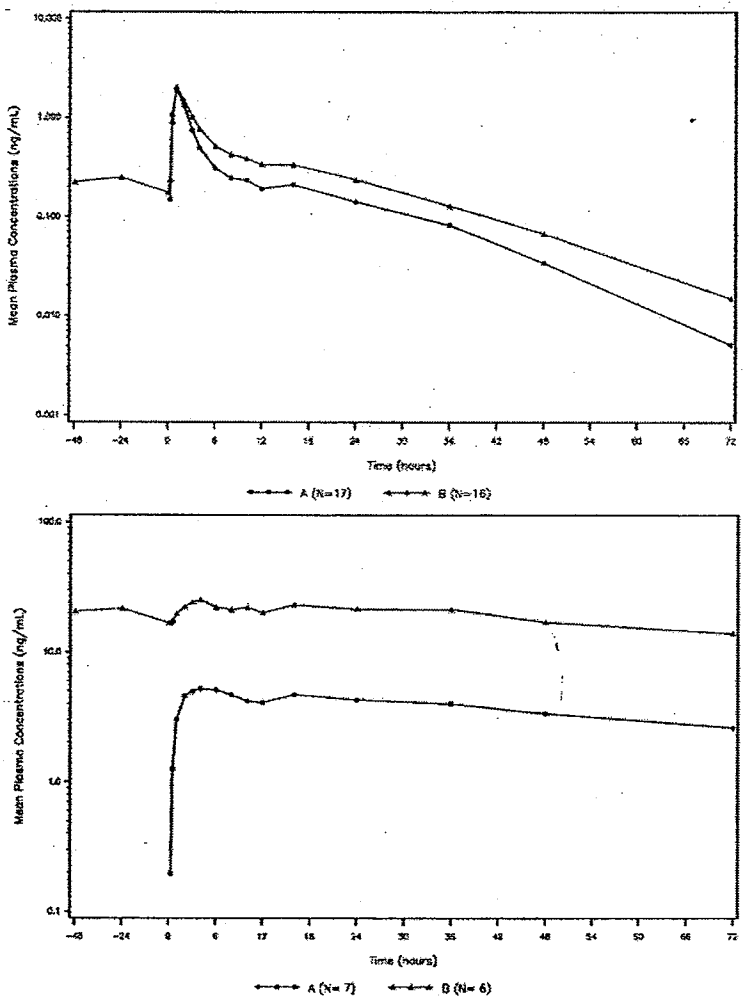

Figure 25: Mean plasma concentrations of l-nebivolol vs. time. Upper Panel, EM, left panel, PM

Table 46: Mean (%CV) Nebivolol glucuronide PK parameters (single dose, Day 1)

Parameter	Extensive Metabolizers (n=17)	Poor Metabolizers (n=7)
AUCL (ng x hr/mL)	318.3 (133.8)	2125 (18.34)
AUCI (ng x hr/mL)	348.4 (154.5)	2345 (18.44)
CPEAK (ng/mL)	45.11 (60.39)	145.1 (21.10)
KEL (hr^{-1})	0.174 (38.47)	0.032 (28.01)
HALF (hr)	5.841 (113.3)	22.98 (22.98)
TPEAK (hr)	2.412 (25.64)	3.571 (14.97)
CL/F[§] (L/hr)	49.99 (41.20)	4.390 (18.39)
Vd/F[§] (L)	294.2 (34.67)	142.6 (20.06)

Table 47: Mean (%CV) Nebivolol glucuronide PK parameters (multiple doses, Day 14)

Parameter	Extensive Metabolizers (EMs) (n=16)	EMs Least Squares Mean Ratio (%)*	EMs 90% Confidence Interval (%)*	Poor Metabolizers (PMs) (n=6)	PMs Least Squares Mean Ratio (%)*	PMs 90% Confidence Interval (%)*
AUCTAU(ngxhr/mL)	433.2 (176.4)	111	104 – 120	2804 (31.73)	117	105 – 130
CPEAK (ng/mL)	52.76 (90.73)	108	96.6 – 120	220.5 (29.97)	153	138 – 170
KEL (hr^{-1})	0.140 (44.09)	86.5	75.9 – 97.1	0.023 (35.67)	69.5	42.9 – 96.1
HALF (hr)	7.427 (120.4)	124	101 – 147	32.81 (28.81)	145	118 – 172
TPEAK (hr)	2.688 (35.22)	110	96.3 – 124	3.667 (14.08)	100	85.4 – 115
CL/F' [¥] (L/hr)	44.31 (43.51)	90.8	82.7 – 99.0	3.893 (33.85)	88.0	78.5 – 97.5
Vd/F' [¶] (L)	325.7 (38.95)	109	93.4 - 124	187.5 (42.61)	133	90.4 - 176
CTROUGH[§] (ng/mL)	6.155 (342.7)	---	---	70.22 (41.15)	---	---
CSS (ng/mL)	18.05 (176.4)	---	---	116.8 (31.73)	---	---
FLUCT1 (%)	395.0 (24.95)	---	---	137.5 (19.93)	---	---
FLUCT2 (%)	3553 (57.75)	---	---	278.4 (38.58)	---	---

* Comparing single-dose parameter values (Day 1) to steady-state parameter values (Day 17). For AUCTAU compared lnAUCTAU to Day 1's

In EM subjects, plasma concentrations reached peak at about 1.3 hours for d-, l-, d,l-nebivolol, and at 2.4 hours for nebivolol glucuronide. The peak plasma concentration of d-isomer (1.1 ng/mL) was 2-fold lower than of l-isomer (2.1 ng/mL), and nebivolol glucuronide peaked at 45 ng/mL. In the PM subjects, plasma concentrations reached peak at about 4 hours for d-, l-, d,l-nebivolol, and nebivolol glucuronide. The PM subjects had 3-4-fold larger mean CPEAK value relative to EM subjects for each of the measured entity. The mean AUCI values for PM subjects were roughly 17, 41, 32, and 7 times larger for d- l-, d, l- nebivolol, and nebivolol glucuronides, respectively, than that observed with the EM subjects.
Figure 26 shows nebivolol glucuronides plasma concentration vs. time profiles.

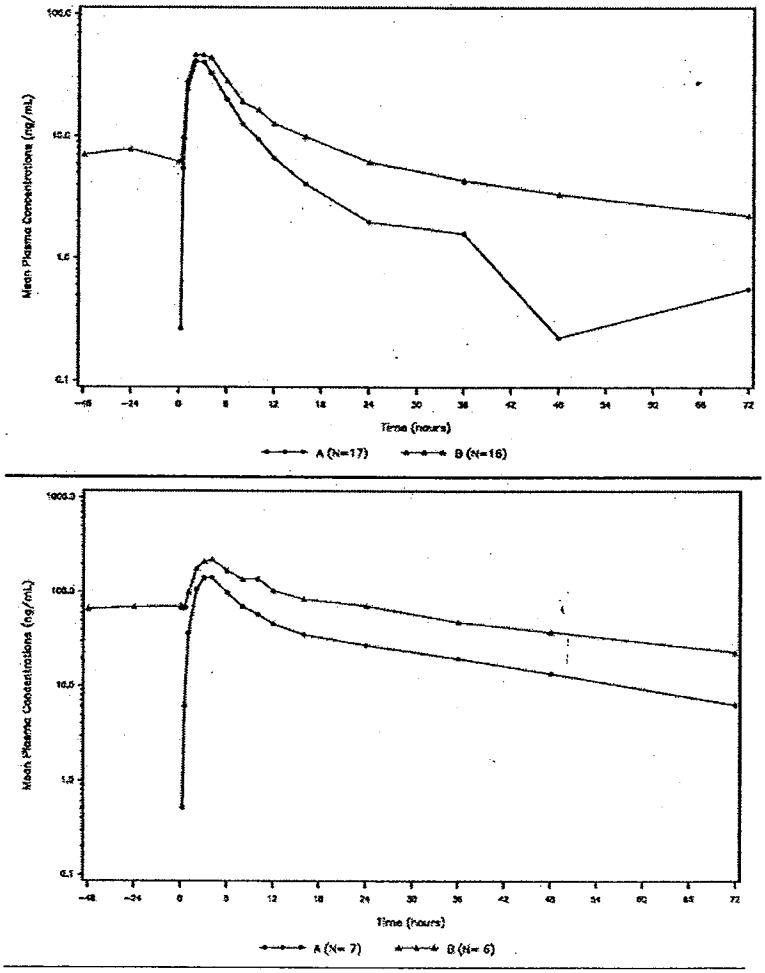

Figure 26: Mean plasma concentrations of nebivolol glucuronide vs. time. Upper panel, EM, low panel, PM

Under steady state conditions, the mean time to peak plasma concentrations (TPEAK) for EM subjects was roughly 1.3 hours following study drug administration for d-, l-, and d, l- nebivolol, and it was 2.7 hours for nebivolol glucuronides. The mean TPEAK for PM subjects was 3.8, 5.7, 3.7, and 3.7 hours following drug administration for d-, l-, d, l- nebivolol, and nebivolol glucuronides, respectively. The PM subjects have 5, 11, 9, and 4- fold higher mean CPEAK value for d-, l-, d, l-nebivolol, and nebivolol glucuronides, respectively, as compared to EM subjects. In PMs, mean AUCTAU values that were 14- fold, 43- fold, 32- fold, and 6- fold greater than in EMs. The mean half-lives for EMs were 13, 17, 13, and 7 hours for d-, l-, d, l-nebivolol, and nebivolol glucuronides, respectively. For PM subjects, the mean half-lives were 22, 73, 56, and 33 hours, respectively.

Steady State vs. Single Dose Comparison

In EM subjects, there were no statistically significant differences between log transformed AUCI from the single dose Day 1 and AUCTAU from the steady state, as well as CPEAK and the non

log transformed pharmacokinetic parameters (KEL, HALF, CL/F, Vd/F) for the d, l- nebivolol moiety (Table 40).
All calculated 90% confidence intervals were between 80% - 125%, except for TPEAK) between single dose or steady state conditions in the EM group, except for TPEAK.
The accumulation indexes for EM and PM subjects were 1.3 and 3.7 (d, l-nebivolol). In the PM group, the CPEAK values were over 3-fold higher under steady- state conditions. The least squares means ratio between the single dose log transformed AUCI and the steady state log transformed AUCTAU was 117% (90% CI 98, 126). The steady state half-life increased 28% compared to single dose. The LSM ratios for other pharmacokinetic parameters were between 80% and 125%, however, the 90% CIs were skewed. Nevertheless, the sponsor concluded that the differences between single dose and steady state d,l-nebivolol pharmacokinetics were not statistically significant and "slight differences could be explained by the low number of PM subjects (N=6)". The fluctuation of d,l-nebivolol plasma concentrations around the Css values were 460% for EMs and 50% for PMs.
In EMs, LSM ratios for AUCTAU, CPEAK, and TPEAK of glucuronides were between 80% and 125%, and the differences were not statistically significant (90% CIs values). The half-life estimates of 5.8 hours after single dose and 7 hours at steady state seem unreasonably small (less than the half-life for the parent drug) most likely due to incomplete blood samples collection and failure to fully characterize the elimination profile of the glucuronides.
In PMs, AUCTAU and TPEAK values of glucuronides at steady state were not statistically different from the same parameters obtained after single dose. CPEAK values were 53% higher at steady state than after the single dose. The same comment regarding incomplete blood sampling for nebivolol glucuronides plasma concentration is valid for PM subjects; therefore, comparison of kel, half-lives, CL/F, Vd/F could not be performed. The fluctuation of nebivolol glucuronides plasma concentrations around the Css were 400% for the EMs and 140% for the PMs.

COMMENTS:

1. The mean single 10 mg dose PK parameters for d-, l-nebivolol and nebivolol glucuronide were similar to the same parameters calculated in the other Phase 1 studies for both EM and PM subjects.
2. The sponsor compared the pharmacokinetic parameters of the sum of d- and l-nebivolol after single and multiple doses. In the EM subjects, there were no statistically significant differences between the d,l-nebivolol pharmacokinetic parameters calculated after single dose and at steady state. In the PM group, the 90% confidence interval for the ratio of single dose and steady state PK parameters for d,l-nebivolol (except for CPEAK and half-life) were skewed but still within 80 to 125%. The steady state half-life was 28% larger compared to single dose. The CPEAK values were over 3-fold higher under steady state conditions in comparison with the single dose value. Although the sponsor explains this increase by low number of PM subjects (N=6), it could be also attributed to the non-linear kinetics of nebivolol. The fluctuations of d,l-nebivolol plasma concentrations around Css were very large in EM subjects (460%).
3. This reviewer compared the d- and l-nebivolol parameters after single and multiple dose. In EMs, the PK parameters of d- and l-nebivolol did not change significantly

(90% CIs were in the range of 80 to 125%). In PMs, CPEAK of d- and l-nebivolol increased by 55% and 355%, respectively. AUClast increased by 97% and TPEAK increased by 31% only for l-nebivolol with no changes for d-nebivolol. The mean AUClast values were more than 80% of the mean AUCI values for d-nebivolol and were about 54% for l-nebivolol pointing out that the parameters estimated for l-nebivolol, particularly, the accumulation ratio value may be unreliable. Since d-nebivolol has β-adrenoceptor properties, and the changes in its parameters were not significant except for only CPEAK, it is not expected that the there is an accumulation of nebivolol in PM subjects.

4. The pharmacokinetic profile of nebivolol glucuronides was not completely characterized and comparison of kel, half-lives, CL/F, Vd/F performed by the sponsor is not valid. The least squares mean ratios for AUCTAU and TPEAK for both EM and PM subjects were not statistically different. After repeated administration of nebivolol, CPEAK values in EMs were not changed, while in PMs CPEAK values increased by 53%. The fluctuations of nebivolol glucuronides plasma concentrations around Css values were very large (400%, EMs and 150%, PMs).

4.2.5 The plasma protein binding and distribution in blood of rac- nebivolol and of its two enantiomers in rats, dogs and humans.

DRUG STUDIED:	**Nebivolol Hydrochloride racemate Janssen Research Foundation Batch No. 612 and No. 653**
INVESTIGATOR:	**G. Mannens,**
STUDY SITE:	**Dept. of Drug Metabolism and PK and Dept. of Toxicology, Turnhoutseweg 30, B-2340 Beerse, Belgium**
DATES OF STUDY:	**May 1994**

OBJECTIVES:

To determine the plasma protein binding and the blood- to- plasma distribution of nebivolol and its separate enantiomers in humans.

METHODS:

Blank human blood and plasma and specifically ditritiated 3H-d, l-nebivolol, 3H-d-nebivolol, and 3H-l-nebivolol were used for the experiments. The in vitro plasma protein binding of nebivolol and its separate enantiomers was investigated by equilibrium dialysis. The plasma protein binding was studied as a function of the drug concentration, the plasma protein concentration, and the plasma pH. The protein binding in the presence of the other enantiomers and the binding to purified human plasma proteins was also investigated. Quantification was done by radioactivity measurements of the ditritiated drugs.

RESULTS:

The plasma protein binding of nebivolol of d- and l- nebivolol averaged 98.13% and 97.85%, respectively. In addition, the plasma protein binding of one enantiomer was not significantly altered in the presence of the other enantiomer and the binding of both enantiomers was independent of the drug concentration up to 100ng/mL. Changes in pH influenced the binding of both enantiomers, at higher pH the binding of both d- and l-nebivolol was increased. In the pH range of 7.0 to 7.7, the binding of d- nebivolol increased from 96.17% to 98.51% and that of l-nebivolol from 95.36% to 98.46%. The investigation of the binding to purified human plasma proteins showed that the enantiomers were predominately bound to human serum albumin (HSA). The binding to HSA at a normal physiological concentration of 4.3% was 99.29% for d-nebivolol and 98.91% for l-nebivolol. The binding to purified α1-acid glycoprotein (AAG) at a normal physiological concentration of 0.07% was 74.14% for d- nebivolol and 71.53% for l-nebivolol.

The blood to plasma concentration ratio (Cb/Cp) of d- and l-nebivolol at 1 ng/mL averaged 1.11 and 1.28, respectively (Table 48).

Table 48: Mean (± SD) distribution of d- nebivolol and l- nebivolol in blood of healthy subjects at a concentration of 1ng/mL.

PK Parameter	*d*-nebivolol	*l*-nebivolol
Blood/plasma concentration ratio	1.11 ± 0.05	1.28 ± 0.05
Distribution (%) in blood to - plasma water - plasma proteins - blood cells	 1.01 ± 0.03 53.2 ± 2.6 45.8 ± 2.6	 1.06 ± 0.09 47.8 ± 2.9 51.1 ± 3.0

The Cb/C ratio of l- nebivolol was not altered in the presence of d- nebivolol and the Cb/C ratio of d- nebivolol was slightly decreased in the presence of the l-enantiomer. In addition, the Cb/C ratio of the racemate averaged the ratios of the separate enantiomers. The percentage of d-and l-nebivolol distributed into blood cells averaged 45.8% and 51.1%, respectively. The mean percentage of d- and l-nebivolol bound to plasma proteins in whole blood was 53.2% and 47.8%, respectively. Only 1% of d- and l- nebivolol was present in plasma water (Table 6.6- 08). The distribution of l- nebivolol in human blood was not altered in the presence of the d-enantiomer and the percentage of d- nebivolol distributed to plasma proteins and to blood cells was slightly altered in the presence of l- nebivolol.

COMMENT:

The plasma protein binding and the distribution of nebivolol enantiomers in blood was stereoselective. d-Nebivolol was more bound to plasma proteins and less distributed to blood cells. However, these differences appear to be very minor.

4.2.6 A Phase I, Open Label Study Investigating the Effects of Hepatic Impairment on the Single Dose Pharmacokinetics of Nebivolol Hydrochloride (NEBI- 0124)

DRUG STUDIED: **Nebivolol Hydrochloride 5mg Tablets**
(5mg of free base nebivolol)
Mylan Pharmaceuticals Inc. Lot # R1H1181

INVESTIGATOR AND STUDY SITE: Robert J. Noveck, M. D., Ph. D.

ANALYTICAL SITE: **Mylan Pharmaceuticals Inc. Bioanalytical Department**
3711 Collins Ferry Road Morgantown, WV 26505
DATES OF STUDY: **Clinical Period: September 12, 2001 - November 4, 2001**
Analytical Phase: June 17-20, 2002 (d- and l-nebivolol)
April 7- May 7, 2003 (conjugated and non-conjugated nebivolol)

OBJECTIVES:
To determine the effect of moderate hepatic impairment on the single dose pharmacokinetics of d-nebivolol, l-nebivolol and, to the extent possible, the major active metabolites of nebivolol hydrochloride.

STUDY DESIGN:
This was an open-label, one-period, single-dose study. Sixteen (16) subjects, eight patients with moderate hepatic impairment and eight healthy volunteers matched according to age, gender, weight, and smoking habit were enrolled from the general population.
Cytochrome P450 2D6 genotyping was performed, but was not utilized as an entrance or matching criteria. Subjects were housed from the evening prior to dosing until 48 hours after dosing. After a supervised fast (at least 4 hours) subjects received a single, oral 5mg (1 x 5mg) dose of nebivolol HCL tablets administered with 240 mL of water at ambient temperature. Lunch and dinner were given 4 and 10 hours after dosing, respectively. Water was given 1 hour and 15 minutes before dosing and 1 hour after dosing. Water was not permitted from 1 hour prior to dosing until 1 hour after dosing, but was allowed at all other times.
Serial blood samples, 10mL (1 x 10mL), were collected at pre- dose and at 0.25, 0.50, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 14, 24, 36, 48, 72, 96 and 120 hours post- dose. Plasma was extracted, divided into 2 aliquots, and stored in suitably labeled tubes at - 70 ° C ± 15 ° C.

ASSAY:
The method for the analysis of d-nebivolol and l-nebivolol in human plasma (heparin) was performed using high performance liquid chromatography with tandem mass spectrometric detection.

Table 49: Assay Characteristics for d- and l-Nebivolol

Parameter	Measure		Reviewer Comment
	Assay (Curve III)		
Linearity	0.04ng/mL to 3.0ng/mL		Satisfactory
Precision (CV %)	d-nebivolol ≤ 6.0	l-nebivolol ≤ 6.0	Satisfactory
Accuracy Between day	d-nebivolol between –5.0% and 5.8%	l-nebivolol between -3.8% and 6.4%	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.

Table 50: Assay for non-conjugated plus conjugated nebivolol

Parameter	Measure	Comment
Linearity	linear from 1.0ng/mL to 800ng/mL	Satisfactory
Precision (CV %)	≤ 6.6	Satisfactory
Accuracy Between day	between –3.1 and 6.0%	Satisfactory
LLOQ	1.0ng/mL	Satisfactory
Specificity		Satisfactory

Chromatograms were shown.
Protein binding was assessed via equilibrium dialysis was performed by . The assay (HPLC) performed on the dialyzed samples was linear For the dialyzed plasma samples, the assay was linear for nebivolol from 100pg/mL to 10000pg/mL. The limit of quantification for this method was and 100pg/mL for the dialyzed human plasma. The between- day precision of the assay in this study was 6.0% or less for nebivolol in dialyzed plasma. The between- day accuracy for this study varied within – 10.5% and 14.8% of the nominal concentration for in the dialyzed plasma.
The representative chromatograms were shown.

PHARMACOKINETICS:
Individual plasma concentrations of d- nebivolol and l-nebivolol were added together at each individual blood collection time point for each subject in order to provide an estimate of the d, l-nebivolol plasma concentration. Single dose pharmacokinetic parameters for d-nebivolol, l-nebivolol, d, l- nebivolol, and nebivolol glucuronides were calculated using noncompartmental techniques.
The individual plasma concentrations for d- nebivolol and l- nebivolol were subtracted from the corresponding conjugated plus non- conjugated nebivolol plasma concentrations to arrive at the

individual nebivolol glucuronides plasma concentrations. Single dose pharmacokinetic parameters were computed using noncompartmental techniques.
The apparent clearance (CL/F for nebivolol and CL/F' for nebivolol glucuronides) was calculated as Dose/AUCI. F represents the oral bioavailability of nebivolol, while F' refers to the fraction of the bioavailable dose of nebivolol systemically converted to nebivolol glucuronides.
All of the parameters calculated for d-, l-, and d, l- nebivolol were also calculated for nebivolol glucuronides. The dose utilized for the apparent clearance and dose normalized parameters was equal to the dose of d, l- nebivolol administered.

STATISTICS

Statistical analyses were performed on the plasma concentrations and the pharmacokinetic parameters for all analytes analyzed using the General Linear Models Procedure (PROC GLM) of SAS Software (SAS Institute, Cary, NC). ANOVA analyses were conducted to test for statistically significant differences between healthy and hepatic impaired subjects and to determine the ratios of the pharmacokinetic parameters using Least Squares Means. The model tested for treatment effects in the parameter means at an alpha level of 0.05. The parameters: AUCL, AUCI, CPEAK, TPEAK, KEL, half- life, CL/F, and Vd/F were analyzed statistically using the non-transformed data. The natural log transformed parameters: LNAUCL, LNAUCI and LNCPEAK were also analyzed. One of the assumptions of the ANOVA analysis is that the groups have equal variances. This was not the case for this study with regards to CL/F, CL/F', Vd/F, and Vd/F'. Therefore, log transformation of these parameters was performed in order for a proper ANOVA analysis to be executed.

RESULTS:

Sixteen subjects (8 moderately hepatically impaired and 8 healthy matched subjects) were enrolled and completed the study. The demographic data are shown in Table 51.

Table 51: Demographic Data

Subject/ Patient #	Age (Yrs)	Sex	Hepatic/ Healthy Match	Height (in)	CYP2D6 (alleles)	Ethnic Background	Dosing Date	Group	Smoking Status	Body Weight (lbs) (Screening)
(0001)	50	F	N/A	64	EM (*1/*1)	B	9/13/2001	Moderate	Yes	191.8
(0002)	64	M	N/A	71	EM (*1/*1)	W	9/13/2001	Moderate	No	246
(0003)	46	F	N/A	65	EM (*1/*4)	B	9/13/2001	Moderate	No	225
(0004)	48	M	N/A	71	EM (*1/*1)	B	9/13/2001	Moderate	No	199
(0005)	58	M	N/A	66	EM (*1/*1)	W	9/13/2001	Moderate	No	194.8
(0006)	46	F	N/A	64.5	EM (*1/*1)	W	9/18/2001	Moderate	No	136.5
(0008)	41	F	3	63	EM (*1/*1)^	B	9/25/2001	Healthy	No	230
(0009)	51	M	4	70	EM (*1/*1)	B	9/25/2001	Healthy	No	184.6
(0010)	53	F	1	64.5	EM (*1/*1)	B	10/9/2001	Healthy	No	194
(0011)	54	M	5	70.5	EM (*1/*4)	W	10/2/2001	Healthy	No	214
(0012)	46	F	6	60	EM (*1/*1)	W	10/2/2001	Healthy	No	132
(0013)	55	M	2	74	EM (*1/*1)	W	10/3/2001	Healthy	No	224.8
(0014)	62	M	N/A	69	ND	W	10/9/2001	Moderate	No	250.8
(0015)	45	M	N/A	71	EM (*1/*1)	W	10/17/2001	Moderate	No	239
(0016)	58	M	14	70	EM (*1/*1)	W	10/18/2001	Healthy	No	273.4
(0017)	47	M	15	74	EM (*1/*1)	W	10/30/2001	Healthy	No	249.2

One healthy subject (#16) had the pharmacokinetic parameters similar to the poor metabolizers (PM). CYP2D6 PM can be excluded based on the test performed with approximately 95% certainty. The remaining 5% must be attributed to the fact that not all known alleles have been tested and unknown alleles may exist; however for the population in question (Caucasian) the test performed covers approximately 95% of all today known alleles. The sponsor included his data in the statistical analysis.
The example of the statistical analysis performed for d,l-nebivolol is shown in Table 52. The variability obtained with the inclusion of the outlier was very high.

Table 52: parameters of d,l-nebivolol, healthy subjects

---------- status=Healthy treat=A ----------

Subject	match	Cpeak (ng/mL)	Tpeak (hr)	AUCl (ng*hr/mL)	AUCi (ng*hr/mL)	Kel (1/hr)	Half (hr)
8	C	0.465	1.000	0.799	0.862	0.6873	1.010
9	D	0.945	1.000	2.279	2.455	0.2839	2.440
10	A	1.407	1.000	4.037	4.318	0.1676	4.140
11	E	1.383	1.000	6.493	.	.	.
12	F	1.634	1.000	7.362	8.092	0.0575	12.050
13	B	0.922	1.000	1.824	2.306	0.0975	7.110
16	G	2.463	6.000	56.128	57.239	0.0441	15.720
17	H	0.804	2.000	2.319	2.958	0.0719	9.630
N		8.000	8.000	8.000	7.000	7.0000	7.000
Mean		1.253	1.750	10.155	11.176	0.2014	7.443
STD		0.618	1.753	18.718	20.441	0.2299	5.353
CV		49.358	100.146	184.320	182.905	114.1400	71.926
Max		2.463	6.000	56.128	57.239	0.6873	15.720
Median		1.164	1.000	3.178	2.958	0.0975	7.110
Min		0.465	1.000	0.799	0.862	0.0441	1.010

The reviewer requested an additional data analysis to compare the hepatic impaired group versus the pooled healthy subjects from NEBI-0125 and the 5-mg dose strength results from NEBI-0126 (dose-proportionality study). The results of these comparisons are included in Tables below (Table 53-Table 56).

Table 53: Mean (%CV) *d*-Nebivolol Pharmacokinetic Parameters In Twelve Healthy And Eight Moderate Hepatic Impaired Subjects Following A Single, Oral 5mg (1 x 5mg) Dose Of Nebivolol Hydrochloride Tablets Under Fasting Conditions

Treatment	AUCL (ng x hr/mL)	AUCI (ng x hr/mL)	CPEAK (ng/mL)	TPEAK (hr)	KEL (hr^{-1})	HALF (hr)	Vd/F (L)	CL/F (L/hr)
A = Healthy	1.909 (70.76)	2.334 (62.90)	0.526 (48.55)	1.292 (53.38)	0.1129 (65.11)	8.461 (52.18)	14452 (46.61)	1489 (57.11)
B = Hepatic Impaired	21.47 (98.45)	22.96 (93.28)	1.703 (77.38)	1.500 (50.40)	0.0439 (80.34)	21.66 (44.90)	4667 (42.43)	206.9 (81.26)

P-value (hepatic vs. healthy)	<0.0001^	<0.0001^	0.0004^	0.532	0.024	0.0006	<0.0001^	<0.0001^

^ Used Natural Log Transformed Parameter to obtain normally distributed data for statistical analysis

Table 54: Mean (%CV) *l*-Nebivolol Pharmacokinetic Parameters In Twelve Healthy And Eight Moderate Hepatic Impaired Subjects Following A Single, Oral 5mg (1 x 5mg) Dose Of Nebivolol Hydrochloride Tablets Under Fasting Conditions

Treatment	AUCL (ng x hr/mL)	AUCI (ng x hr/mL)	CPEAK (ng/mL)	TPEAK (hr)	KEL (hr^{-1})	HALF (hr)	Vd/F (L)	CL/F (L/hr)
A = Healthy	4.161 (34.34)	5.029 (30.77)	1.020 (48.52)	1.292 (53.38)	0.0425 (41.26)	18.14 (27.71)	14296 (44.92)	550.7 (36.31)
B = Hepatic Impaired	24.48 (90.00)	27.42 (85.35)	2.415 (64.00)	1.375 (37.64)	0.0255 (62.45)	33.32 (37.34)	5890 (53.71)	136.3 (55.65)
P-value (hepatic vs. healthy)	<0.0001^	<0.0001^	0.002^	0.775	0.041	0.001	0.001^	<0.0001^

^ Used Natural Log Transformed Parameter to obtain normally distributed data for statistical analysis

Table 55: Mean (%CV) *d,l*-Nebivolol Pharmacokinetic Parameters In Twelve Healthy And Eight Moderate Hepatic Impaired Subjects Following A Single, Oral 5mg (1 x 5mg) Dose Of Nebivolol Hydrochloride Tablets Under Fasting Conditions

Treatment	AUCL (ng x hr/mL)	AUCI (ng x hr/mL)	CPEAK (ng/mL)	TPEAK (hr)	KEL (hr –1)	HALF (hr)	Vd/F (L)	CL/F (L/hr)
A = Healthy	**6.246 (42.66)**	**6.888 (42.19)**	**1.546 (47.92)**	**1.292 (53.38)**	**0.0634 (54.41)**	**12.82 (32.19)**	**15347 (52.97)**	**851.3 (42.48)**
B = Hepatic Impaired	**46.25 (93.05)**	**48.99 (91.87)**	**4.101 (69.16)**	**1.500 (50.40)**	**0.0370 (56.09)**	**22.18 (35.40)**	**4547 (49.14)**	**168.2 (63.90)**
P-value (hepatic vs. healthy)	**<0.0001^**	**<0.0001^**	**0.0009^**	**0.532**	**0.069**	**0.003**	**<0.0001^**	**<0.0001^**

^ Used Natural Log Transformed Parameter to obtain normally distributed data for statistical analysis

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Table 56: Mean (%CV) Nebivolol Glucuronides Pharmacokinetic Parameters In Twelve Healthy And Eight Moderate Hepatic Impaired Subjects Following A Single, Oral 5mg (1 x 5mg) Dose Of Nebivolol Hydrochloride Tablets Under Fasting Conditions

Treatment	AUCL (ng x hr/mL)	AUCI (ng x hr/mL)	CPEAK (ng/mL)	TPEAK (hr)	KEL (hr^{-1})	HALF (hr)	Vd/F (L)	CL/F (L/hr)
A = Healthy	106.5 (43.89)	118.8 (40.29)	20.13 (31.10)	2.000 (42.64)	0.2143 (21.61)	3.388 (23.27)	223.6 (27.27)	47.89 (35.60)
B = Hepatic Impaired	341.9 (66.32)	358.2* (71.42)	26.89 (44.13)	2.625 (28.34)	0.0798* (87.74)	15.06* (65.09)	325.1* (43.72)	22.95* (74.49)
P-value (hepatic vs. healthy)	0.0009^	0.003^	0.179^	0.109	<0.0001	0.0006	0.052^	0.003^

* n = 7 subjects for this parameter due to the inability to determine the apparent elimination rate constant in Subject 4 from NEBI-0124.
^ Used Natural Log Transformed Parameter to obtain normally distributed data for statistical analysis

In these Tables CL/F was apparent clearance and Vd/F was apparent volume of distribution.

Moderately hepatic impaired individuals exhibit a statistically significant increase in the unbound fraction of nebivolol in plasma as compared to their healthy matched counterparts. The least squares mean for the percentage of unbound nebivolol in a healthy individual in plasma was 1.90%, while for a moderate hepatic impaired subject, the percent of unbound nebivolol in plasma was 2.45%. This corresponds to a statistically significant ($p < 0.05$) increase in the percent of unbound nebivolol circulating in the plasma in a moderately hepatic impaired individual relative to a healthy subject.
These results may indicate the need to decrease the initial dose of nebivolol when administering nebivolol to patient with moderate hepatic impairment.

COMMENTS

1. One healthy subject (#16) had the pharmacokinetic parameters similar to the poor metabolizers (PM). The sponsor originally included his data in the statistical analysis. The FDA consider that CYP2D6 PM can be excluded based on the test performed with approximately 95% certainty. The remaining 5% must be attributed to the fact that not all known alleles have been tested and unknown alleles may exist; however for the population in question (Caucasian) the test performed cover approximately 95% of all today known alleles. The contradictory finding (2D6 EM with PM phenotype) cannot be explained by the data provided by the sponsor. Co-medications or other covariates (including genetic ones) can be the cause. Therefore, this subject was considered as an outlier and additional data analysis was performed to compare healthy subjects from the studies 0124, 0125, and 0126 (N=12) with the hepatic impaired patients.

2. The results of this data analysis show that the difference between groups was statistically significant in all parameters; however, the early conclusions of the sponsor have changed. These changes should be reflected in the Package Insert.

3. Compared to the healthy subjects, the exposure to d,l-nebivolol (AUCI) in patients with moderate hepatic impairment increased 7-fold, half-life increased 1.7 times and the apparent clearance decreased by 80%. The exposure to nebivolol glucuronides (AUCI) in patients with moderate hepatic impairment increased 13-fold, half-life increased 4.4 times and the apparent clearance decreased by 52%. The comparison of nebivolol glucuronides parameters should be interpreted with caution because the parameters reflect the behavior of the mixture of many compounds whose pharmacokinetic characterization was not complete for the healthy subjects at low nebivolol dose of 5 mg due to assay limitation.

The pharmacokinetic of nebivolol in severely impaired patients have not been studied.

RECOMMENDATION

The Agency recommends to contraindicate the use of nebivolol in severely hepatic impaired patients.

The Package Insert should adapt the following language for the CLINICAL PHARMACOLOGY Section, Special Population, Hepatic Disease:

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4.2.7 A Phase I, Open- Label Study Investigating the Effects of Renal Impairment on the Single Dose Pharmacokinetics of Nebivolol Hydrochloride (NEBI-125)

DRUG STUDIED: **Nebivolol HCL 5 mg Tablets (5mg of free base nebivolol)**
Mylan Pharmaceuticals Inc. Lot # R1H1181
Manufacture Date: April 17, 2000

INVESTIGATORS: **Raymond Vargas, M. D., Gary Matzke, Pharm. D.**

STUDY SITES:

ANALYTICAL SITE: **Mylan Pharmaceuticals Inc.**
Bioanalytical Department
3711 Collins Ferry Road Morgantown, WV 26505

DATES OF STUDY: **Clinical Phase: 11/07/2001 - 2/23/2003**
Analytical Phase: April 10, 2003 – May 2, 2003

OBJECTIVES:
To determine the effect of renal impairment on the single dose pharmacokinetics of d-nebivolol, l-nebivolol and, to the extent possible, the major active metabolites of nebivolol hydroehloride.

STUDY DESIGN:
This was an open- label, one- period, single- dose study. Twenty- one (21) patients, divided into 3 groups with mild, moderate, or severe renal impairment, classification based upon the Cockroft- Gault equation for estimating creatinine clearance or a 24 hour urine creatinine clearance determination, and five (5) healthy volunteers, approximately matched to the mean value of the renal impaired groups for the following parameters: age, gender, weight, and smoking habit, were enrolled from the general population. Cytochrome P450 2D6 genotyping was performed, but was not utilized as an entrance or matching criteria. Subjects were housed from the evening prior to dosing until 48 hours after dosing. After a supervised fast (at least 4 hours) subjects received a single, oral 5mg (1 x 5mg) dose of nebivolol HCL tablets administered with 240 mL of water at ambient temperature. Lunch and dinner were given 4 and 10 hours after dosing, respectively. Water was given 1 hour and 15 minutes before dosing and 1 hour after dosing. Water was not permitted from 1 hour prior to dosing until 1 hour after dosing, but was allowed at all other times.
Serial blood samples were collected within 30 minutes prior to dosing (2 x 10mL) and the following times relative to dosing (6 x 10mL): 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0, 14, 24, 48, 72, 96 and 120 hours.

ASSAY:
The assay utilized two different standard curve ranges, one for EMs and another for PMs. The method for the analysis of d-nebivolol and l-nebivolol in human plasma (heparin) was performed using high performance liquid chromatography with tandem mass spectrometric detection.

Table 57: Assay Characteristics for d- and l-Nebivolol

Parameter	Measure		Reviewer Comment
	Assay for Extensive Metabolizers (Curve III)		
Linearity	0.04ng/mL to 3.0ng/mL		Satisfactory
Precision (CV %)	d-nebivolol ≤ 6.1	l-nebivolol ≤ 5.7	Satisfactory
Accuracy Between day	d-nebivolol between –4.4% and 5.6%	l-nebivolol between -3.8% and 5.1%	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 (Table below).

Table 58: Assay for non-conjugated plus conjugated nebivolol.

Parameter	Measure	Comment
Linearity	linear from 1.0ng/mL to 800ng/mL	Satisfactory
Precision (CV %)	≤ 7.4	Satisfactory
Accuracy Between day	between –5.4 and 1.8%	Satisfactory
LLOQ	1.0ng/mL	Satisfactory
Specificity		Satisfactory

Chromatograms were shown.

PROTEIN BINDING

The samples underwent equilibrium dialysis and the dialyzed samples were assayed using HPLC with tandem mass spectrometric detection. The assay was linear for nebivolol from [redacted]. For the dialyzed plasma samples, the assay was linear for nebivolol from 100pg/mL to 10000pg/mL. The method developed for the determination of nebivolol in dialyzed human plasma [redacted] was performed using high performance liquid chromatography with tandem. The limit of quantification for this method was [redacted] 100pg/mL for the dialyzed human plasma. The between- day precision of the assay in this study was [redacted] 2.9% or less for nebivolol in dialyzed plasma. The between- day accuracy for this study varied within [redacted] – 6.8% and 2.3% of the nominal concentration for nebivolol in the dialyzed plasma.

PHARMACOKINETICS:

Individual plasma concentrations of d-nebivolol and l-nebivolol were added together at each individual blood collection time point for each subject in order to provide an estimate of the d, l-nebivolol plasma concentration. Single- dose pharmacokinetic parameters for d-nebivolol, l-

nebivolol, d, l-nebivolol, and nebivolol glucuronides were calculated using noncompartmental techniques.
The individual plasma concentrations for d-nebivolol and l-nebivolol were subtracted from the corresponding conjugated plus non- conjugated nebivolol plasma concentrations to obtain the individual nebivolol glucuronides plasma concentrations. Statistical analyses were then performed on the individual plasma concentrations for nebivolol glucuronides. Single dose pharmacokinetic parameters were computed using non- compartmental techniques.

RESULTS:

Twenty- six male and female subjects between the ages of 30 and 79 completed this study.
The demographic data are shown in Table 59.

Table 59: Demographic Data

Subject/ Patient	Age (Yrs)	Sex	Genotype CYP2D6 (Allele)	Ethnic Background	Height (in)	Dosing Date	Group	Smoking Status	Body Weight (lb) (Day -1)
(0002)	69	M	EM (*1/*1)	Black	66	11/16/01	Mild	Yes	144
(0005)	59	F	EM (*1/*1)	Black	66	11/08/01	Moderate	Yes	120.6
(0006)	47	M	EM (*1/*4)	White	67	11/08/01	Severe	No	156.2
(0007)	71	M	EM (*1/*5)	White	68	11/27/01	Mild	Yes	161.2
(0008)	53	M	EM (*1/*1)	White	72	11/16/01	Moderate	No	170.2
(0009)†	61	M	EM (*1/*1)	Black	64	11/16/01	Mild	No	157.8
(0011)†	45	F	EM (*1/*1)	Black	64	11/27/01	Moderate	Yes	151.6
0012)†	65	F	EM (*1/*4)	White	57	12/12/01	Moderate	No	148.8
0014)†	40	F	EM (*1/*1)	Black	62	12/12/01	Moderate	No	147.8
(0016)†	50	M	EM (*1/*1)	Black	71	12/27/01	Mild	No	215.2
(0018)†	51	F	EM (*1/*1)	White	64	1/08/02	Healthy	No	152.4
(0019)	66	M	EM (*1/*1)	Black	69	1/16/02	Moderate	No	173.8
(0020)†	79	F	EM (*1/*1)	Black	64	1/30/02	Moderate	No	154.
(0022)	78	M	EM (*1/*1)	Black	66	2/06/02	Moderate	No	124
(0023)	55	M	EM (*1/*4)	White	64	2/26/02	Healthy	No	152
0024)†	51	M	EM (*1/*4)	White	72	7/19/02	Severe	No	285
(0025)	66	M	PM (*3/*4)¥	White	65	8/23/02	Healthy	No	131.8
(0027)	72	M	EM (*1/*2)¥	White	70	9/24/02	Healthy	No	167.8
(0028)	46	M	EM (*1/*1)	White	72	12/03/02	Healthy	Yes	179
0029)†	51	M	EM (*1/*1)	White	66	12/03/02	Mild	No	188
(0030)	58	M	EM (*1/*1)	Black	69	2/18/03	Severe	Yes	174.4

One subject from the healthy group was genotyped as PM, his pharmacokinetic parameters were not included in the statistical assessments.
Tables below (Table 60-Table 62) provide the summary PK parameters for d-nebivolol, l-nebivolol and d, l-nebivolol in EM and PM subjects. Figures 1-4 display the plasma concentrations profiles for d-nebivolol, l-nebivolol and d, l-nebivolol in EM and PM subjects for each treatment.

Table 60: Mean (CV) d-Nebivolol PK parameters

Parameter	A = Healthy (n = 4)	B = Mild Renal Impaired (n = 7)	C = Moderate Renal Impaired (n = 9)	D = Severe Renal Impaired (n = 5)	Ratio B vs A^	Ratio C vs A^	Ratio D vs A^
AUCL (ng x hr/mL)	1.752 (39.11)	1.413 (37.87)	3.769 (79.92)	9.812 (93.50)	81.7	170	400
AUCI (ng x hr/mL)	2.305 (41.43)	1.740 (42.10)	5.403 (63.50)	10.84 (87.51)	76.3	210	364
CPEAK (ng/mL)	0.705 (47.53)	0.589 (44.55)	0.731 (53.77)	0.849 (41.99)	85.4	93.8	123
TPEAK (hr)	0.875 (28.57)	1.000 (0.000)	2.333 (74.23)	1.800 (46.48)	----	----	----
KEL (hr^{-1})	0.130 (82.17)	0.299 (66.58)	0.088 (51.64)	0.065 (65.54)	----	----	----
HALF (hr)	7.532 (48.86)	3.506 (66.21)	9.708 (44.95)	14.44 (51.85)	----	----	----
Vd/F¶ (L)	11641 (34.99)	6914 (42.03)	7562 (31.41)	6615 (52.97)	----	----	----
Cl/F§ (L/hr)	1329 (60.94)	1689 (47.96)	695.9 (67.97)	446.0 (77.49)	----	----	----

Table 61: Mean (CV) l-Nebivolol PK parameters

Parameter	A = Healthy (n = 4)	B = Mild Renal Impaired (n = 7)	C = Moderate Renal Impaired (n = 9)	D = Severe Renal Impaired (n = 5)	Ratio B vs A^	Ratio C vs A^	Ratio D vs A^
AUCL (ng x hr/mL)	4.039 (37.81)	2.606 (31.14)	5.618 (62.94)	12.01 (90.97)	65.8	120	224
AUCI (ng x hr/mL)	5.090 (35.68)	3.508 (47.42)	7.329 (54.71)	13.40 (84.70)	67.3	129	208
CPEAK (ng/mL)	1.308 (51.60)	0.943 (39.35)	1.128 (54.37)	1.178 (32.86)	75.4	77.9	95.2
TPEAK (hr)	0.875 (28.57)	1.000 (0.000)	2.000 (82.92)	1.600 (55.90)	----	----	----
KEL (hr^{-1})	0.057 (43.28)	0.138 (133.8)	0.047 (21.44)	0.039 (54.69)	----	----	----
HALF (hr)	14.07 (43.40)	11.63 (83.54)	15.48 (21.73)	21.58 (43.29)	----	----	----
Vd/F¶ (L)	10430 (34.17)	10900 (44.56)	9972 (57.81)	7976 (50.31)	----	----	----
Cl/F§ (L/hr)	567.1 (50.91)	842.0 (42.78)	507.6 (83.58)	330.5 (73.45)	----	----	----

Table 62: Mean (CV) d,l-Nebivolol PK parameters

Parameter	Treatment									
	A = Healthy (n = 4)	B = Mild Renal Impaired (n = 7)	C = Moderate Renal Impaired (n = 9)	D = Severe Renal Impaired (n = 5)	Ratio B vs A^	Ratio C vs A^	Ratio D vs A^	Confidence Interval B vs A^	Confidence Interval C vs A^	Confidence Interval D vs A^
AUCL (ng x hr/mL)	5.970 (38.41)	4.155 (30.25)	9.574 (68.51)	22.13 (90.64)	71.3	136	278	34% - 149%	67% - 275%	127% - 612%
AUCI (ng x hr/mL)	6.593 (37.94)	4.551 (32.64)	11.28 (64.02)	23.36 (88.69)	70.2	147	268	34% - 147%	70% - 307%	122% - 592%
CPEAK (ng/mL)	2.013 (49.92)	1.532 (40.99)	1.848 (53.96)	1.998 (34.66)	79.0	83.1	104	43% - 147%	46% - 150%	54% - 202%
TPEAK (hr)	0.875 (28.57)	1.000 (0.000)	2.000 (82.92)	1.600 (55.90)	----	----	----	----	----	----
KEL (hr^{-1})	0.096 (47.34)	0.228 (81.90)	0.085 (44.13)	0.068 (62.69)	----	----	----	----	----	----
HALF (hr)	8.349 (40.13)	5.083 (67.06)	9.205 (31.86)	14.07 (57.25)	----	----	----	----	----	----
Vd/F¶ (L)	9409 (25.58)	7485 (44.17)	7654 (43.99)	5704 (42.48)	----	----	----	----	----	----
Cl/F§ (L/hr)	891.1 (53.52)	1241 (44.34)	737.8 (90.39)	416.3 (78.12)	----	----	----	----	----	----

Table 63: Mean (CV) nebivolol glucuronides parameters.

Parameter	A = Healthy (n = 4)	B = Mild Renal Impaired (n = 7)	C = Moderate Renal Impaired (n = 9)	D = Severe Renal Impaired (n = 5)	Ratio B vs A^	Ratio C vs A^	Ratio D vs A^
AUCL (ng x hr/mL)	105.7 (50.57)	137.7 (29.82)	282.8 (71.87)	735.1 (96.10)	138	231	489
AUCI (ng x hr/mL)	117.6 (50.07)	148.2 (30.62)	324.9 (66.74)	761.7 (93.39)	133	245	462
CPEAK (ng/mL)	20.38 (33.33)	20.82 (8.023)	29.69 (48.51)	43.31 (62.75)	106	133	188
TPEAK (hr)	1.500 (38.49)	2.143 (17.64)	3.444 (54.53)	3.000 (33.33)	----	----	----
KEL (hr^{-1})	0.198 (31.59)	0.145 (33.41)	0.112 (42.04)	0.081 (90.72)	----	----	----
HALF (hr)	3.730 (25.58)	5.214 (29.21)	7.405 (47.24)	13.67 (56.97)	----	----	----
Vd/F'¶ (L)	252.8 (27.72)	257.5 (19.15)	210.9 (67.98)	186.1 (45.87)	----	----	----
Cl/F'§ (L/hr)	51.08 (48.61)	36.54 (30.44)	23.99 (76.07)	16.87 (116.4)	----	----	----

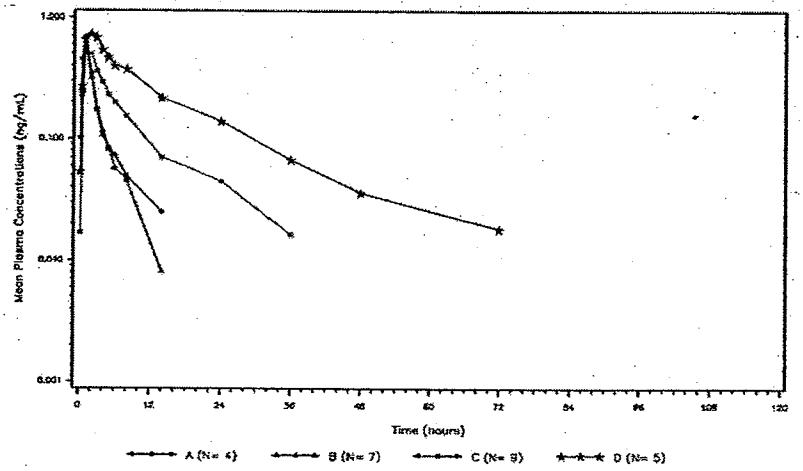

Figure 27: Mean Plasma concentrations of d-nebivolol vs. time.

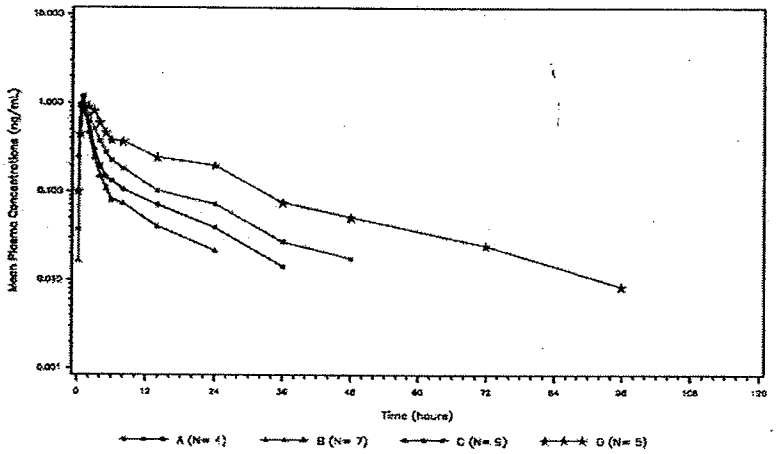

Figure 28: Mean Plasma concentrations of l-nebivolol vs. time.

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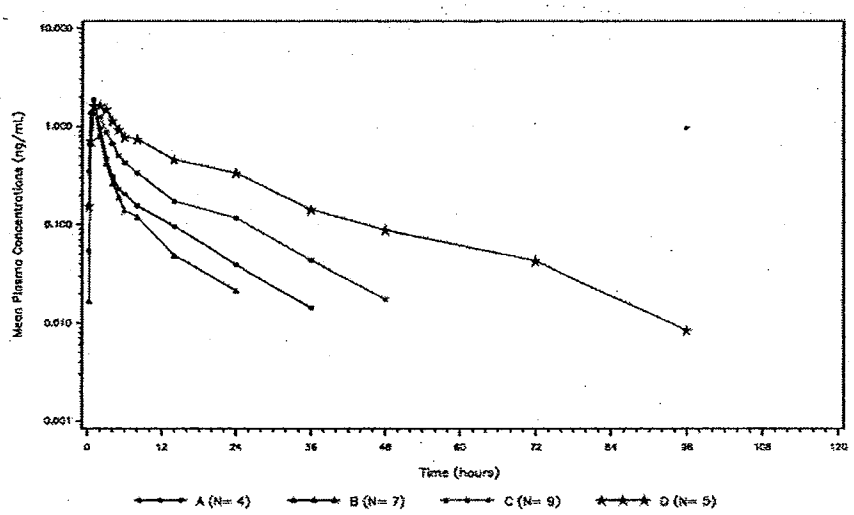

Figure 29: Mean Plasma concentrations of d,l-nebivolol vs. time

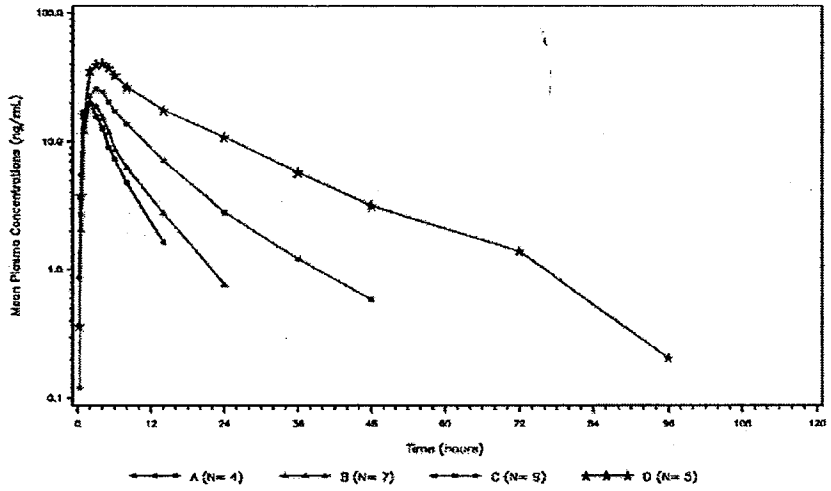

Figure 30: Mean Plasma concentrations of nebivolol glucuronides vs. time.

Based on the results shown in the Tables 61-64, the sponsor concluded that the pharmacokinetic parameters observed in this study for the healthy group were similar to values seen previously following a 5-mg dose of nebivolol in NEBI-0126 study. Both isomers, d-nebivolol and l-nebivolol were affected by renal impairment without any preference.

The mean CL/F was reduced approximately 48%, 10%, and 17% in moderate renal impaired patients for d-, l-, and d, l- nebivolol, respectively while for severe renal impaired subjects, the mean CL/F decreased 66%, 42%, and 53%, respectively. None of these decreases in CL/F for moderate and severe renal impaired subjects were statistically significant (p-value > 0.05). For nebivolol glucuronides, the mean CL/F' values were decreased by 28%, 53%, and 67% for the mild, moderate, and severe renal impairment groups, respectively and these decreases were statistically significant (p-value < 0.05).

PROTEIN BINDING

The resulting least squares mean for the percentage of unbound nebivolol in plasma for healthy subjects was 1.79% (ANOVA). Similar results were obtained in the study NEBI- 0124. The resulting least squares mean for the percentage of unbound nebivolol in plasma was 2.12% for mild renal impaired subjects, 2.11% for moderate renal impaired subjects, and 2.02% for the severe renal impaired subjects. None of percentage of unbound nebivolol values for the renal impaired groups or the renal impaired subjects taken as a whole were found to be statistically different from their healthy volunteer counterparts.

All renal impaired subjects were extensive CYP2D6 metabolizers (EMs). Four of the five healthy volunteers were EMs, while the other subject was a poor CYP2D6 metabolizer (PM).

COMMENTS:

1. This was a study comparing the pharmacokinetic of d-, l-, d,l-nebivolol and nebivolol glucuronides in healthy volunteers and in patients with mild, moderate and severe renal impairment. The sponsor adequately characterized the pharmacokinetic of each of the nebivolol components in the patients with renal impairment and made proper recommendations in the PI.
2. The pharmacokinetics of nebivolol in healthy subjects was characterized poorly. The estimation of half-life of 7.5 hours (range of 2.4 and 11 hours) was based on data obtained up to 14 hours (d-nebivolol) and the half-life of 14 hours (range of 8.7 and 20 hours) was based on data collection up to 24 hours (l-nebivolol) in 4 subjects. In study NEB-0126, plasma concentrations for d- and l-nebivolol were measured up to 48 hours and half-lives were estimated as 9 and 20 hours. Therefore, estimation of AUCI based on k_{el} is not reliable. As a result, the AUCL values for both d- and l-nebivolol in normal subjects and mild, and moderate renally impaired patients represent 70-80% of the AUCI values.
3. The comparison of AUCL values between groups cannot be made due to the different time of blood sample collection (for example, range of 14 and 72 hours for d–nebivolol).
4. If compared to the values calculated for the healthy subjects in studies NEBI126 and NEBI127, the decrease in clearance of d-nebivolol was 30% in the patients with moderately impaired renal function and 55% in patients with severely impaired renal function. The decrease in clearance of l-nebivolol was 34% for patients with severe renal impairment. Since the drug is administered as a racemic mixture, the recommendations for the patients with renal impairment is related to d,l-nebivolol. The mean CL/F of d, l- nebivolol was reduced by 17% in moderate renal impaired patients, and by 53% in severe renal impaired patients most probably due to high variability in the pharmacokinetic parameters, these changes were not statistically significant (p-value > 0.05). For nebivolol glucuronides, the mean CL/F' values were decreased by 28%, 53%, and 67% for the mild, moderate, and severe renal impairment groups, respectively and these decreases were statistically significant (p-value < 0.05).
5. [redacted]

6. There were no statistically significant differences in protein binding between the renal impaired subjects and their healthy matched counterparts.

PI:

4.2.8 Absorption, Metabolism, and Excretion of Nebivolol in Healthy Male Volunteers after a Single Oral Dose of 15mg 14C- Nebivolol HCL (NEBI- 0136)

DRUG STUDIED: **^{14}C-Nebivolol Hydrochloride, 15mg**
Mylan Pharmaceuticals Inc. Lot No.: X1020: 22A

INVESTIGATOR: **Dr. David Hoelscher**

STUDY SITE:

ANALYTICAL SITE:

DATE OF STUDY: **Clinical Period: July 27, 2001 – August 14, 2001**
Analytical Phase: July 20, 2001 – August 14, 2001

OBJECTIVES:

To determine the absorption, metabolism, and excretion of nebivolol after a single oral dose of 100 μ Ci/15mg 14C- nebivolol HCL in healthy male subjects characterized according to their CYP2D6 metabolizing status.

STUDY DESIGN:

This was a single- dose, open- label, mass balance, Phase I clinical study in healthy subjects (three CYP2D6 extensive and three poor metabolizers). The radiolabeled 14C- nebivolol HCL (120.9mg and total radioactivity of 727.17 μCi) was used to prepare the 14C oral solution. Subjects fasted for 10 hours prior to and 4 hours after dosing. On Day 1, each subject drank a 60mL dosing solution which contained approximately 100 μCi/15mg (free base equivalent) 14C-nebivolol HCL and 180mL of water.

The blood samples were collected on Day 1 prior to dosing and at 0.5, 1, 2, 4, 6, 9, 15, 24, 36, 48, 72, 96, and 168hr post-dose. Urine and feces was collected 15 minutes prior to dosing and thereafter over the following intervals: 0 to 6, 6- 12, 12- 24, 24- 48hr post- dose and for each subsequent 24- hour period for the duration of the study (up to 336 hours post- dose for EM and 432 hours post- dose for PM).

ASSAY:

Samples of blood, plasma, urine, and fecal homogenate were analyzed in triplicate after they were oxidized to $^{14}CO_2$ gas and captured with a [redacted] oxidizer.

PHARMACOKINETICS:

The pharmacokinetic estimates of radioactivity were obtained by a noncompartmental method. Descriptive statistics (means and standard deviations) were calculated for the pharmacokinetic variables. No statistical comparison was made between EM and PM due to the limited number of subjects.

RESULTS:

Six subjects (3 EM and 3 PM) were enrolled and completed the study. The demographic data are shown in Table 64.

Table 64: Demographic Data

Subject Number	CYP2D6 Genomic Status (Alleles)	Age	Sex	Race[a]	Height (in)	Frame Size	Entry Weight (lb)	Exit Weight (lb)
1	EM (*1*1)	45	M	H	69.50	M	182.50	192.00
2	EM (*1*1)	38	M	B	71.75	M	192.75	188.00
3	EM (*1*1)	24	M	W	68.75	S	164.25	159.00
4	PM (*4*4)	51	M	W	71.75	M	187.75	185.20
5	PM (*4*4)	57	M	W	71.25	L	190.50	189.00
6	PM (*4*4)	29	M	W	73.00	M	171.00	174.00

Figures below (Figure 31 and Figure 32) show the mean whole blood and plasma nebivolol concentration of radioactivity vs. time for extensive and poor metabolizers.

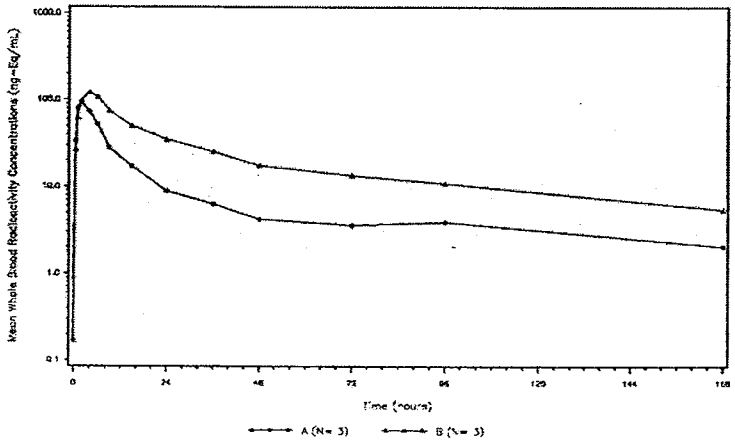

Figure 31: Mean Concentration of Radioactivity in Whole Blood for EM and PM Subjects following Administration of a Single 15mg Dose of 100 μ Ci of 14C- Nebivolol HCL

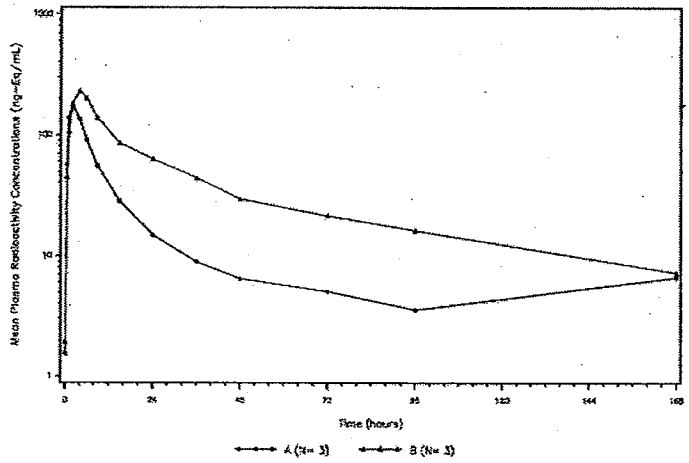

Figure 32: Mean Concentration of Radioactivity in Plasma for EM and PM Subjects following Administration of a Single 15mg Dose of 100 μ Ci of 14CNebivolol HCL

Table 65summarizes the individual and mean pharmacokinetic parameters of total radioactivity in whole blood and plasma samples as nebivolol equivalents.

Table 65: Individual and mean PK parameters estimated based on total radioactivity in plasma and whole blood after a single oral dose of 15 mg 14C-nebivolol

Subject No.	Total Radioactivity in Whole Blood*				Total Radioactivity in Plasma*			
	CPEAK (ng-Eq/mL)	TPEAK (hr)	AUCL (ng-Eq*hr/mL)	HALF (hr)	CPEAK (ng-Eq/mL)	TPEAK (hr)	AUCL (ng-Eq*hr/mL)	HALF (hr)
01								
02								
03								
Mean ± SD	92.8 ± 6.9	2	1334.9 ± 89.6	86.1 ± 66.0	173.8 ± 9.0	2	2247.4 ± 403.2	44.0
04								
05								
06								
Mean ± SD	121.2 ± 16.4	4	3453.8 ± 866.8	73.1 ± 8.9	229.4 ± 33.9	4	5836.9 ± 1522.0	60.1 ± 6.0

The total radioactivity in whole blood was lower than equivalent measurements in plasma. The maximum radioactivity levels in blood and plasma were attained at 2 hours in all EM and at 4 hours for the PM. The observed average CPEAK values were 30% higher in PM in comparison with EM subjects. Mean AUCL values were approximately 2.6- fold greater in PM, compared with EM, with respective values of 3453.8 and 1334.9ng- Eq* hr/mL in blood, and 5836.9 and 2247.4ng- Eq* hr/mL in plasma. The t1/2 estimates were incomplete and cannot be used for comparison.

Preferential distribution of radioactivity to plasma versus blood was observed with mean blood-to- plasma total radioactivity ratios of 0.53 (range: 0.50- 0.57) and 0.60 (range: 0.47- 0.76) for

CPEAK and AUCL in all subjects, respectively. Previous data reported a blood- to- plasma nebivolol concentration ratio of 1.11 in human blood when measured in vitro.
The sponsor explained the lower blood- to- plasma total radioactivity ratios by the preferential binding of the metabolites of nebivolol to plasma protein compared to erythrocytes.

The dose recovery in urine and feces is shown in Figure 33. The sponsor did not have any comments about this recovery.

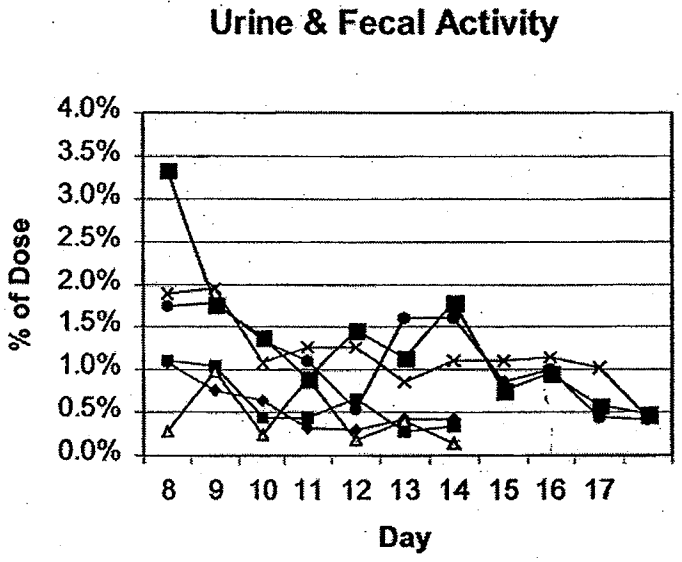

Figure 33: Nebivolol ng-equivalents per mL of urine and feces

COMMENTS:

The maximum radioactivity levels in blood and plasma were attained at 2 hours in EM and at 4 hours for PM. The total radioactivity in blood or plasma were 30 % higher in PM when compared to EM subjects and the systemic exposure values based on AUCL differed 2.6- fold between EM and PM subjects. The apparent terminal elimination half-lives (HALF) of total radioactivity obtained from PMs were longer in blood (73.1hr) than that in plasma (60.1hr). The half-life estimations in EMs were not sufficient to make a comparison.

Mean blood- to- plasma total radioactivity ratios of less than 1 were observed for both CPEAK and AUCL. Previous data reported a blood- to- plasma nebivolol concentration ratio of 1.11 in human blood when measured in vitro. According to these controversial finding, it is not clear if nebivolol preferentially distributed into erythrocytes (in vitro data) or preferentially binds to plasma proteins.

The sponsor measured the combined percent of dose recovery in urine and feces. There were no comments in the report regarding this information. The data show that nebivolol radioactivity was measured in EM subjects up to Day 14 post-dose (0.27% total radioactivity recovery) and up to Day 18 in PM subjects (0.43% total radioactivity recovery).

4.2.9 Metabolism of [14C]- Nebivolol in Human: Mass Balance and Metabolite Profiling/Identification in Plasma and Excreta (NEBI-142)

Authors: Zhe-Ming Gu, Ph. D. Robert A. Robinson, Ph. D.
Study Completed: February 14, 2003
Testing Facility:

PROTOCOL NUMBER: NEBI- 0142
DRUG STUDIED: ^{14}C-Nebivolol Hydrochloride, 15mg
Mylan Pharmaceuticals Inc. Lot No.: X1020: 22A

OBJECTIVES:

To evaluate the dose concentration, to determine the mass balance, to investigate the metabolite profiles, and to isolate and characterize/identify, to the extent possible, significant metabolites present in human plasma, urine, and feces following oral administration of a single dose of (±) -[14C]- nebivolol in healthy male subjects.

STUDY DESIGN:

This was a single dose, open label, mass balance, Phase I clinical study in healthy subjects (three CYP2D6 extensive and three poor metabolizers). See Study 0136 review for the description of clinical design and sampling schedule.

METHODS

Test Articles:

Nebivolol hydrochloride, (±) - ([2R*[1S*, 5S*(S*)]]) a, a'-[iminobis(methylene)] bis [6- fluoro- 3,4- dihydro- 2H- 1- benzopyran- 2- methanol] hydrochloride, Lot Nos.:

Radiolabeled:	X1020: 4A (Pre- Isotopic Dilution)
	X1020: 22A (Post- Isotopic dilution)
Non- radiolabeled:	ZR067555PUA391
Radiochemical Purity:	for X1020: 22A
Specific Activity:	2.62 μ Ci/μ mol

Reference Standards:

d- Nebivolol (Batch No. R067138)

l- Nebivolol (Batch No. R067145)

6- Fluoro- 2-{ 2-[2-(6- fluoro- chroman- 2- yl)- 2- hydroxy- ethylamino]- 1- hydroxyethyl} chroman- 4- ol (4- OH Nebivolol) (Batch No. RD- NEBDER- 005055)

6- Fluoro- 2-{ 2-[2-(6- fluoro- chroman- 2- yl)- 2- hydroxy- ethylamino]- 1- hydroxyethyl} chroman- 5- ol (5- OH Nebivolol) (Batch No. RD- NEBDER- 005087)

6- Fluoro- 2-{ 2-[2-(6- fluoro- chroman- 2- yl)- 2- hydroxy- ethylamino]- 1- hydroxyethyl} chroman- 8- ol (8- OH Nebivolol) (Batch No. RD- NEBDER- 005089)

(6- Fluoro- chroman- 2- yl)- hydroxy- acetic acid (Batch No. RD- NEBDER- 005011)

1-(6- Fluoro- chroman- 2- yl)- ethane- 1,2- diol (Batch No. RD- NEBDER- 006002)

2- Amino- 1-(6- fluoro- chroman- 2- yl)- ethanol (Batch No. RD- NEBDER- 004096)

Total Radioactivity in Plasma, Urine, and in Feces

Total radioactivity (TR) levels were analyzed by direct LSC. TR levels in urine were determined by direct LSC of weighed, duplicate aliquots (ca. 1 g) from each sample. Concentrations of radioactivity in feces were determined by combusting triplicate aliquots (ca. 0.5–0.7 g) of each fecal homogenate followed by LSC. Final TR levels in urine and feces were also expressed as a % of administered dose based on a dose level of 2.0896×10^8 DPM or 94.1 μCi per subject.

Determination of Metabolite Profiles:

The metabolite profiles of 14C- nebivolol in urine and the extracts of plasma and feces were determined by radio- HPLC using the HPLC system and conditions described under HPLC.

Isolation and Purification of Metabolites:

Urine, feces, and plasma samples were fractionated by ___ HPLC and the effluent was collected every 15 seconds into 2- mL 96- well receiving plates. Multiple collections were made for each matrix in order to obtain sufficient quantity of metabolites for subsequent LC/MS and LC/MS/MS analyses (see Study 0136 review).

RESULTS:

Six subjects (3 EM and 3 PM) were enrolled and completed the study (see Study 0136 review).

Radioactivity in Whole Blood and Plasma

For EM subjects, the radioactivity of the maximum concentrations (C_{max}) of 2494 ±129 DPM/mL (equivalent to 174 ±9 ng/mL of nebivolol) at 2 h post-dose. The radioactivity levels were 211 ±75) DPM/mL (equivalent to 15 ±5 ng/mL of nebivolol) by 24 h.

For PM subjects, observed plasma C_{max} of 3292 ± 487 DPM/mL (equivalent to 229 ±34 ng/mL of nebivolol) occurred at 4 h. The radioactivity at 24 h (897 ±278 DPM/mL) and 96 h (231 ± 73 DPM/mL) in PM subjects was much higher than in the EM subjects. The mean radioactivity for each group is shown in Figure 34.

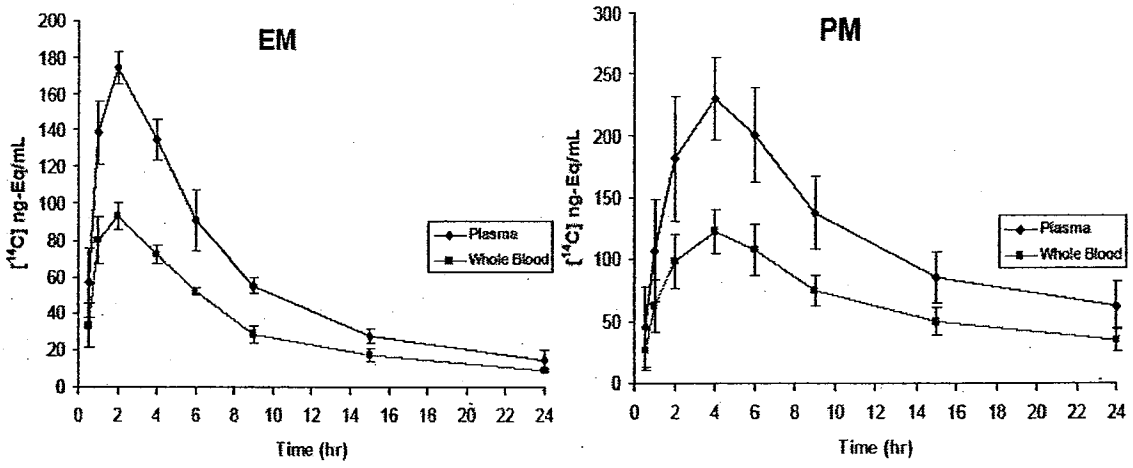

Figure 34: Mean Concentrations of Total Radioactivity in Plasma and Whole Blood for a Period of 0.5–24 h Following a Single Oral Dose of $^{[14}C]$-Nebivolol to EM and PM subjects

Radioactivity in Urine

An average of 38.36% (±6.66%) of the administered dose was excreted in the urine of EM subjects over 14 days following dose administration with individual subject values in a

range of 31.07–44.12%. A higher average value of 66.49% (±1.10%) of the administered dose was excreted in the urine of PM subjects over 18 days with individual subject values equating to 65.23%–67.29% (Table 66).

Table 66: Cumulative Percent of Nebivolol Dose Recovered in Urine

Interval	PM					EM				
(hours)	04-	05-	06-	Mean	SD	01-	02-	03-	Mean	SD
6	5.91%	11.30%	12.56%	9.92%	3.53%	8.63%	16.65%	17.00%	14.09%	4.73%
12	18.62%	20.43%	23.11%	20.72%	2.26%	17.13%	25.96%	24.73%	22.61%	4.78%
24	27.61%	29.86%	31.74%	29.74%	2.07%	20.84%	30.75%	30.43%	27.34%	5.63%
48	38.18%	40.45%	42.13%	40.25%	1.98%	25.56%	36.98%	34.61%	32.38%	6.03%
72	44.27%	46.28%	48.09%	46.21%	1.91%	27.00%	38.84%	35.66%	33.83%	6.13%
96	47.78%	50.37%	51.83%	49.99%	2.05%	27.74%	40.50%	36.85%	35.03%	6.57%
120	50.98%	53.31%	54.69%	52.99%	1.88%	28.57%	40.78%	37.62%	35.66%	6.34%
144	53.44%	55.60%	56.79%	55.28%	1.70%	29.04%	41.60%	38.20%	36.28%	6.50%
168	55.35%	57.39%	58.50%	57.08%	1.60%	29.64%	42.24%	38.57%	36.82%	6.48%
192	56.98%	59.02%	59.88%	58.63%	1.49%	29.89%	42.73%	38.81%	37.14%	6.58%
216	58.32%	60.35%	61.09%	59.92%	1.43%	30.19%	43.10%	39.06%	37.45%	6.60%
240	59.42%	61.47%	62.05%	60.98%	1.38%	30.42%	43.37%	39.32%	37.70%	6.62%
264	60.38%	62.47%	62.92%	61.92%	1.36%	30.63%	43.62%	39.48%	37.91%	6.64%
288	61.25%	63.36%	63.68%	62.76%	1.32%	30.82%	43.84%	39.66%	38.11%	6.65%
312	62.10%	64.20%	64.39%	63.56%	1.27%	30.97%	44.01%	39.78%	38.25%	6.65%
336	62.75%	64.91%	64.99%	64.22%	1.27%	31.07%	44.12%	39.88%	38.36%	6.66%
360	63.45%	65.59%	65.59%	64.88%	1.24%					
384	64.14%	66.21%	66.10%	65.48%	1.16%					
408	64.75%	66.78%	66.54%	66.02%	1.11%					
432	65.23%	67.29%	66.95%	66.49%	1.10%					

More than half of the urine radioactivity was excreted from both subject groups within the first two days post-dose.

Radioactivity in Feces

An average of 43.57% (±23.03%) of the administered dose was excreted in the feces of EM subjects over 14 days following dose administration, with individual values of 53.77%, 17.20%, and 59.75%, respectively, for Subjects 1, 2, and 3. A much lower average value of 13.06% (±4.64%) of the administered dose was excreted in the feces of PM subjects over 18 days following dosing with individual values of, 13.46%, 8.23%, and 17.49% for Subjects 4, 5, and 6, respectively (Table 67).

Table 67: Cumulative Percent of Nebivolol Dose Recovered in Feces

Interval	PM					EM					
(hours)	04	05	06	Mean	SD	01	02	03	Mean of 3[1]	SD	Mean of 2[2]
0 - 6	NS	NS	NS	N/A	N/A	7.37%	NS	NS	7.37%	N/A	7.37%
6 - 12	NS	NS	NS	N/A	N/A	7.37%	NS	5.38%	6.38%	N/A	6.38%
12 - 24	NS	0.41%	3.92%	2.17%	N/A	7.37%	NS	5.38%	6.38%	N/A	6.38%
24 - 48	1.63%	0.41%	3.92%	1.99%	1.78%	16.30%	NS	5.38%	10.84%	N/A	10.84%
48 - 72	4.14%	1.04%	3.92%	3.03%	1.73%	30.43%	0.01%	50.47%	26.97%	25.41%	40.45%
72 - 96	6.24%	1.04%	8.29%	5.19%	3.74%	45.95%	NS	54.83%	50.39%	N/A	50.39%
96 - 120	8.07%	1.04%	10.02%	6.38%	4.72%	49.40%	15.20%	56.38%	40.33%	22.04%	52.89%
120 - 144	9.16%	1.57%	11.54%	7.42%	5.21%	50.32%	15.20%	57.74%	41.09%	22.72%	54.03%
144 - 168	9.45%	2.77%	12.81%	8.34%	5.11%	51.22%	15.98%	58.05%	41.75%	22.58%	54.64%
168 - 192	10.06%	5.13%	13.33%	9.51%	4.13%	51.72%	16.30%	58.05%	42.02%	22.50%	54.89%
192 - 216	10.59%	5.81%	13.97%	10.12%	4.10%	52.22%	16.30%	58.82%	42.45%	22.88%	55.52%
216 - 240	10.99%	6.29%	14.83%	10.70%	4.28%	53.19%	16.52%	58.82%	42.84%	22.97%	56.01%
240 - 264	11.33%	6.29%	15.35%	10.99%	4.54%	53.27%	16.84%	59.35%	43.15%	22.99%	56.31%
264 - 288	12.02%	6.93%	15.35%	11.43%	4.24%	53.45%	17.07%	59.35%	43.29%	22.90%	56.40%
288 - 312	12.02%	7.37%	15.80%	11.73%	4.22%	53.57%	17.13%	59.75%	43.48%	23.03%	56.66%
312 - 336	12.35%	7.75%	16.51%	12.20%	4.38%	53.77%	17.20%	59.75%	43.57%	23.03%	56.76%
336 - 360	12.67%	7.75%	16.72%	12.38%	4.49%						
360 - 384	13.20%	7.97%	17.25%	12.81%	4.65%						
384 - 408	13.37%	8.07%	17.25%	12.90%	4.61%						
408 - 432	13.46%	8.23%	17.49%	13.06%	4.64%						

[1] Mean value of all three EM subjects (NS time point was not included in mean calculation).
[2] Mean value of Subjects 1 and 3 (NS time point was not included in mean calculation).

A majority of fecal radioactivity was excreted within the first 7 days post-dose for both subject groups. A much lower dose (17.20%) was recovered in feces from Subject 2 compared to the other two EM subjects due to incomplete sample collection. Figure 2 shows the urinary and fecal excretion in both groups.

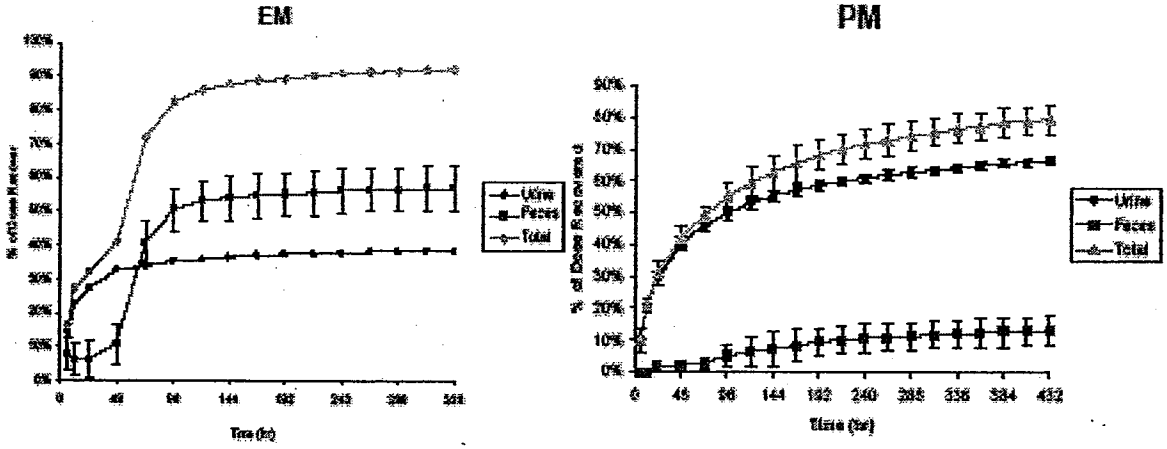

Figure 35: Mean Concentrations of Total Radioactivity (TR) in Urine and Feces After a Single Oral Dose of [^{14}C]-Nebivolol to EM and PM subjects

Within-group metabolite radio-profiles were very similar for both EM and PM groups, 0-168 h collections were prepared and analyzed by radio-HPLC for metabolite distribution. Figures below (Figure 36- Figure 38) show metabolite radio-profiles for pooled EM and PM in plasma, urine and feces.

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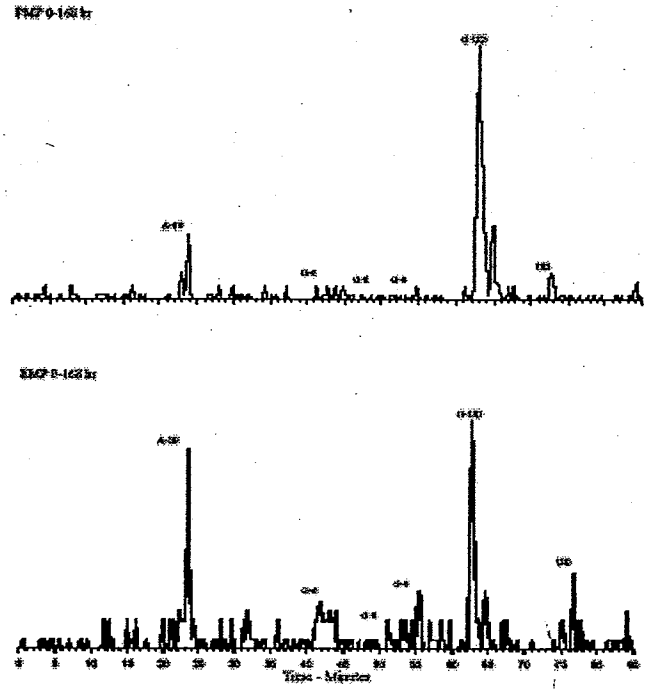

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Figure 36: Comparison of Pooled Plasma Methanol Extracts from Poor and Extensive Metabolizers

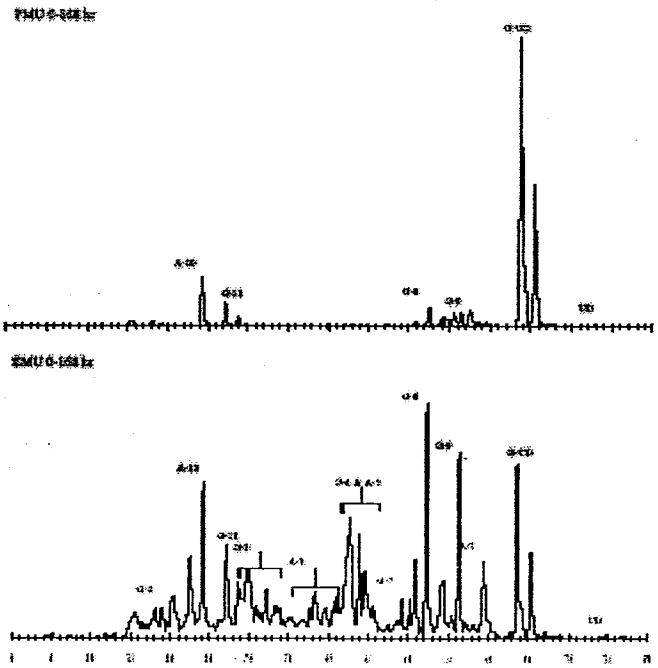

Figure 37: Comparison of Pooled Urine Methanol Extracts from Poor and Extensive Metabolizers

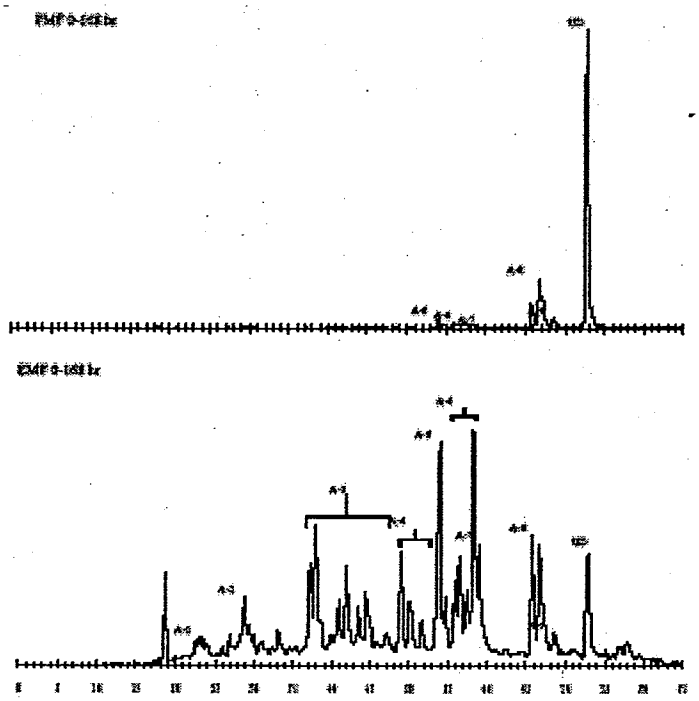

Figure 38: Comparison of Pooled Feces Methanol Extracts from Poor and Extensive Metabolizers

Metabolites were classified as either free nebivolol metabolites, designated with codes A-1 to A-10, or glucuronide conjugates of nebivolol metabolites, coded as G-1 to G-11. Glucuronide conjugates of the unchanged drug were also coded as G-UD.

The structural formulas, molecular weight and codes of the metabolites found in plasma, urine and feces are shown in Table 68.

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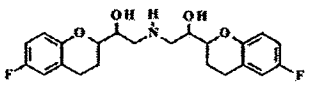
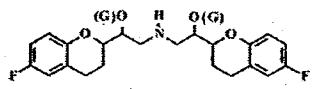
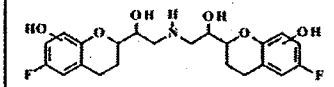
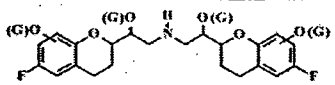
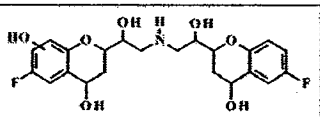
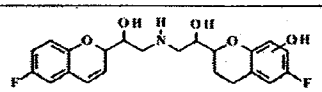
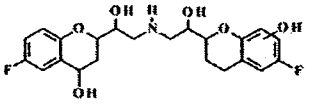
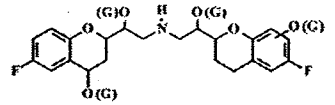
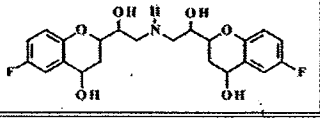
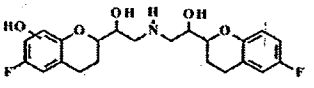
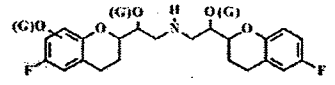
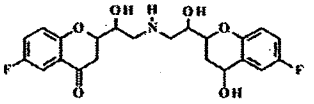
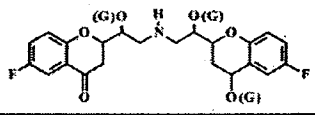
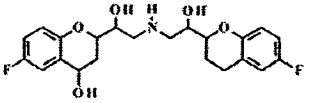
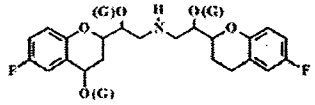
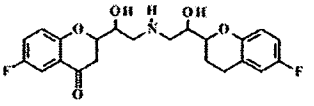
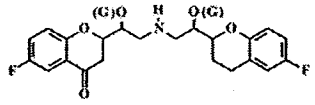
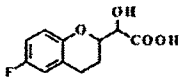
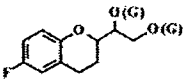
Table 68: Nebivolol metabolites found in plasma, urine and feces

Met. Code No	Met Code Name	MW	Representative Structure	Met. Code No	Met Code Name	MW	Representative Structure
UD	Unchanged Drug (*d,l*-Nebivolol)	405		G-UD	*d,l*-Nebivolol glucuronides	581	
A-1	Diphenol Nebivolols	437		G-1	Diphenol Nebivolol Glucuronides	613	
A-2	Phenolic Diol Nebivolols	453		A-4	Phenolic Dehydro Nebivolols	419	
A-3	Phenolic Monohydroxy Nebivolols	437		G-3	Phenolic Monohydroxy Nebivolol Glucuronides	613	
A-5	Diol Nebivolols	437		G-5	No A-5 conjugate identified		
A-6	Monophenol Nebivolols	421		G-6	Monophenol Nebivolol Glucuronides	597	
A-7	Keto Hydroxy Nebivolols	435		G-7	Keto Hydroxy Nebivolol, Glucuronides	611	
A-8	Mono Hydroxy Nebivolols	421		G-8	Mono Hydroxy Nebivolol Glucuronide	597	
A-9	Mono Keto Nebivolols	419		G-9	Mono Keto Nebivolol Glucuronides	595	
A-10	Dealkylated Carboxylic Acid	226		G-11	Dealkylated Diol Glucuronides	388	

Nebivolol was not observed in EMs plasma after one hour post-dose. In PMs, it was detectable over 96 hours post-dose subjects. Three to five metabolite regions were observed in EM and PM plasma. The majority of the radioactivity in plasma of both EM and PM subjects consisted of nebivolol glucuronides, followed by metabolite A-10. Additional metabolites such as G-1, G-3, A-3, G-6, G-7, G-8-I, G-9, G-8-II, and G-11 were also observed in EM plasma, however, they were generally non-detected in PM plasma except for G-8-I.
Table 69shows the metabolite profile summary in plasma.

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Table 69: Time Course Metabolite Profile Summary in Plasma from EMs and PMs

Mean (ng/mL, Nebivolol Equivalents) Values from Three EM Subjects

Time	G-1	A-10	G-11	G-3	A-3	G-6	G-7	G-8-I	G-9	G-8-II	G-UD	UD
0.5	1.6	3.9	2.6	1.5	1.9	6.9	0.8	2.6	1.3	1.8	21.9	3.8
1	3.3	12.5	2.5	5.5	7.3	10.2	3.3	8.5	4.2	4.2	51.2	3.4
2	3.8	20.5	3.6	9.6	10.5	20.0	5.7	12.3	11.3	4.1	52.9	NA
4	4.0	13.6	4.3	9.4	10.5	18.2	2.8	6.8	10.0	3.2	29.6	NA
6	NA	14.4	3.3	5.7	6.6	12.3	NA	7.0	7.8	2.9	18.2	NA
9	2.1	7.7	1.9	4.7	4.1	6.7	NA	3.2	4.4	1.4	10.9	NA
15	1.0	4.3	1.2	2.9	2.1	3.7	NA	1.3	2.1	NA	4.8	NA
24	0.5	2.5	NA	1.7	1.8	1.8	NA	1.1	1.6	NA	1.7	NA
36-96	NA	0.9	NA	1.0	0.8	0.9	NA	NA	1.1	NA	0.6	NA

*Note: The contribution of metabolite A-7 is not summarized due to minor detectable levels (below the limit of quantitation).

Mean (ng/mL, Nebivolol Equivalents) Values from Three PM Subjects

Time (h)	A-10	G-8-I	G-UD	UD
0.5	4.2	NA	24.4	4.6
1	8.4	2.0	67.5	6.2
2	17.7	2.3	132.6	6.1
4	15.4	3.7	173.0	6.6
6	11.4	3.2	152.6	7.6
9	10.1	2.7	100.6	6.6
15	5.7	2.3	58.5	4.7
24	4.2	1.1	44.1	4.6
36	4.8	1.0	28.8	4.1
48	3.0	0.9	18.9	3.2
72	2.3	1.2	12.8	2.3
96	1.5	0.6	9.3	1.7

**Note: The contributions of metabolites G-8-II, G-9, and G-11 are not summarized due to minor detectable levels (below the limit of quantitation).

Twelve chromatographic metabolite regions are defined in EM urine: G-1 (diphenol nebivolol glucuronides), A-10 (*N*-dealkylated carboxylic acid), G-11 (*N*-dealkylated diol glucuronides) and A-2 (phenolic diol), G-3 (phenolic monohydroxy nebivolol glucuronides), A-3 (phenolic monohydroxy nebivolol) and G-3, G-6 (monophenol nebivolol glucuronides) and A-3, G-7 (keto-hydroxy nebivolol glucuronides), G-8 (monohydroxy nebivolol glucuronides), G-9 (monoketo nebivolol glucuronides), G-8 (additional monohydroxy nebivolol glucuronide region), A-7 (ketohydroxy nebivolols), and G-UD (nebivolol glucuronides). Eight metabolite regions were observed in PM urine including A-10, G-11, G-6, G-8, G 9, A-7, G-8 (additional monohydroxy nebivolol glucuronide region), and G-UD. G-UD is the predominant observed metabolite in PM urine.

In the EM subjects urine, 38.36% of the radioactivity was excreted with 36.82% of the dose accounted for during the first 7 days of collection. Metabolites G-6/A-3 (5.93% of dose), G-8 (5.52% of dose), G-3 (4.53% of dose), A-3/G-3 (3.91% of dose) are the major groups of metabolites followed by G-11/A-2 (2.74% of dose), G-UD (2.66% of dose), G-1 (2.64% of

dose), A-10 (2.12% of dose), G-9 (1.68% of dose), A-7 (1.24% of dose), and G-7 (0.39% of dose).

In the PM subjects urine, 66.5% of the radioactivity was excreted with 57.08% of the dose accounted for over the first 7 days of collection. A majority of the metabolite profile was attributed to unchanged drug glucuronides (G-UD, 34.18% of dose), and monohydroxy nebivolol glucuronide (G-8) that accounted for 7.10% of dose. N-dealkylated acid (A-10) accounted for 4.44% of dose. G-11 (2.35% of dose), G-6, and A-7 were less significant metabolites.

Table 70 shows the metabolites mass-balance (percent recovered dose) in urine.

Table 70: Profiles of Metabolites in Pooled (0-168 hr) Urine (% of Dose)

		EM (Subjects 1-3)		PM (Subjects 4-6)	
% Dose in Pooled Urine:		36.82%		57.08%	
Region	Met. Code	%HPLC	%Dose	%HPLC	%Dose
1	G-1	7.18%	2.64%	ND	NA
2	A-10	5.75%	2.12%	7.78%	4.44%
3	G-11 & A-2	7.45%	2.74%	4.11%	2.35% (G-11 only)
4	G-3	12.31%	4.53%	ND	NA
5	A-3 & G-3	10.61%	3.91%	ND	NA
6	G-6 & A-3	16.11%	5.93%	2.05%	1.17% (G-6 only)
7	G-7	1.06%	0.39%	ND	NA
8	G-8	13.67%	5.03%	8.68%	4.95%
9	G-9	4.56%	1.68%	2.22%	1.27%
10	G-8	1.33%	0.49%	3.77%	2.15%
11	A-7	3.36%	1.24%	1.21%	0.69%
12	G-UD	7.23%	2.66%	59.88%	34.18%
	Subtotal:	90.62%	33.40%	89.70%	51.20%
	Unknown:	9.38%	3.42%	10.30%	5.88%

Ten chromatographic metabolite regions were observed in pooled, EM fecal methanol extracts, including A-1, A-2, A-3, A-4, A-5, A-6, A-7, A-8/A-6, A-9, and UD. There are six metabolite regions found in pooled PM fecal extracts, namely, A-5, A-6, A-7, A-8, A-9, and UD. Approximately 80% of the radioactivity in EM feces extracts was identified as oxidative metabolites and only 4.38% (0.98% of administered dose) of UD was found. Conversely, of the In PMs, 51.12% of feces radioactivity was UD (3.82% of administered dose), while 30% of the radioactivity was comprised of oxidative metabolites.

In EMs, 43.57% of the radioactivity was excreted into the feces with 41.75% of the dose accounted for during the first 7 days of collection. A-3 (4-OH phenolic nebivolols; 6.24% of the dose), A-6 (monophenolic nebivolols; 2.88% of the dose), A-5 (4,4'-dihydroxy nebivolols; 2.40% of the dose) constitute the three major groups followed by A-8/A-6 (monohydroxy/monophenol nebivolols; 1.64% of the dose) and A-4 (dehydrophenol; 1.42% of the dose). The unchanged drug (UD) in EMs feces amounted to ~1% of the dose.

In PMs, 13% of the radioactivity was excreted into the feces with 8.34% of the dose was accounted for in the first 7 days of collection, UD was the major component, 3.82% of the dose. Other metabolites detected included A-8 (monohydroxy nebivolols; 1.44% of the dose), A-6 (monophenolic nebivolols; 0.40% of the dose), A-5 (4,4'-dihydroxy nebivolols; 0.25% of the dose), A-7 (keto-4-OH nebivolols, minor) and A-9 (keto-nebivolols, minor).

Table 71 shows the metabolites mass-balance (percent recovered dose) in urine.

Table 71: Profiles of Metabolites in Pooled (0-168 hr) Feces (% of Dose)

		EM (Subjects 1-3)		PM (Subjects 4-6)	
% Dose in Pooled Feces:		41.75%		8.34%	
		% Distribution	% Dose	% Distribution	% Dose
Methanol Extracts		53.43%	22.31%	89.61%	7.47%
Region	Met. Code	%HPLC	%Dose	%HPLC	%Dose
1	A-1	2.43%	0.54%	ND	NA
2	A-2	4.84%	1.08%	ND	NA
3	A-3	27.98%	6.24%	ND	NA
4	A-4	6.35%	1.42%	ND	NA
5	A-5	10.78%	2.41%	3.29%	0.25%
6	A-6	12.92%	2.88%	5.34%	0.40%
7	A-7	5.45%	1.22%	2.08%	0.16%
8	A-8+A-6	7.33%	1.64%	19.28%	1.44% (A-8 only)
9	A-9	1.72%	0.38%	3.09%	0.23%
10	UD	4.38%	0.98%	51.12%	3.82%
	Subtotal:	84.18%	18.79%	84.20%	6.30%
	Unknown:	15.82%	3.52%	15.80%	1.17%
PES		46.57%	19.44%	10.39%	0.87%

HPLC, LC/MS, and LC/MS/MS Analyses of Test Article and Reference Standards

HPLC UV (chromatograms of reference compounds were provided. The test article and available reference standards were analyzed by LC/MS and MS/MS to obtain fragmentation pattern and retention times. The available LC/MS and/or MS/MS spectral data was used to illustrate a reference standard or isolated metabolite in the same order of the following four traces, i.e., Trace A: representing either a UV or radioactive chromatographic profile, Trace B: depicting a selected ion chromatogram, Trace C: showing a full scan mode mass spectrum, and Trace D: providing a MS/MS spectrum of the adduct molecular ion.

Figure 39 describes the metabolic pathways of nebivolol in EM subjects.

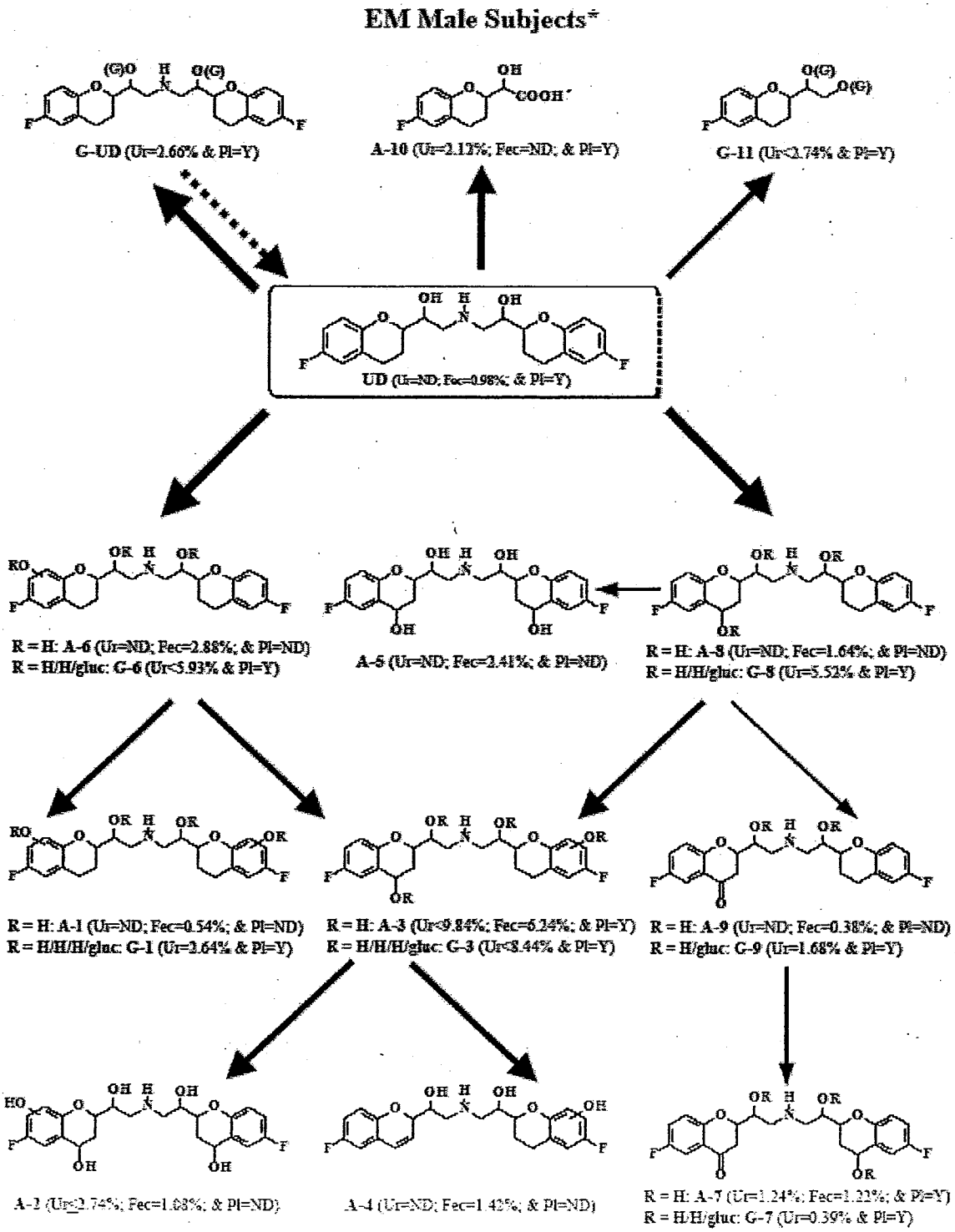

Figure 39: Proposed Metabolic Pathways of Nebivolol Following a Single Oral Dose in Male EM Subjects

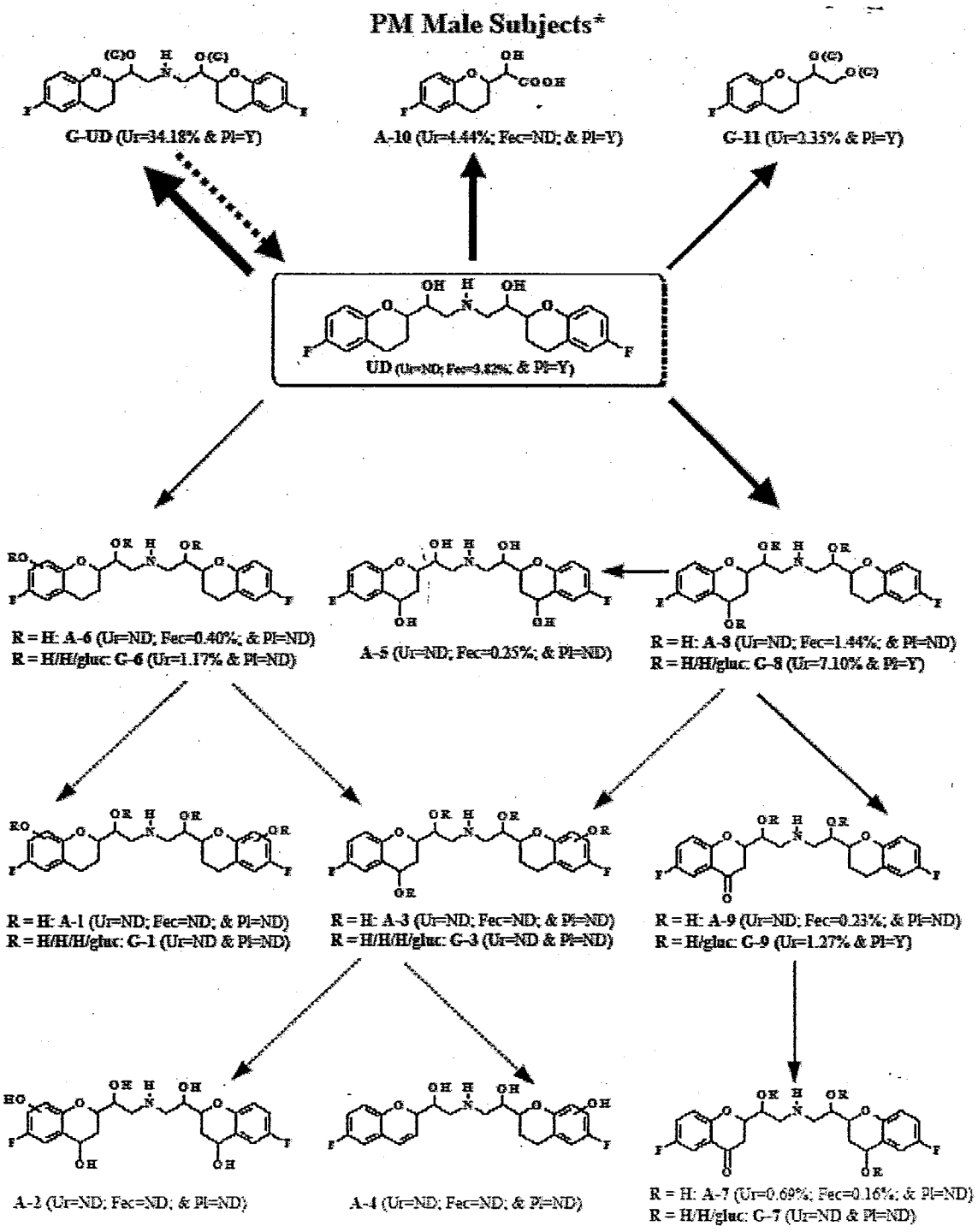

Figure 40: Proposed Metabolic Pathways of Nebivolol Following a Single Oral Dose in Male PM Subjects

COMMENTS:

1. The mass balance study performed by the sponsor accounted for the nebivolol distribution and excretion in 3 EM and 3 PM subjects. Nebivolol was quickly absorbed and extensively metabolized in human subjects following a single oral dose of [^{14}C]-nebivolol HCL.
2. The sponsor satisfactorily described the metabolic pathways of d,l-nebivolol in 3 EM and 3 PM subjects. The major metabolic pathways included formation of glucuronides of unchanged drug, mono- to multiple-hydroxylations on alicyclic and/or aromatic rings followed by glucuronidation, and formation of N-dealkylated derivatives. Glucuronidation of d-nebivolol occurred much more rapidly than on the l-enantiomer. The ratio of d-nebivolol glucuronide/l-nebivolol glucuronide in urine was 80:20.
3. The observed Tmax of total radioactivity in plasma was 2 h and 4 h for EM and PM subjects, respectively. In plasma, most of the metabolites detected were glucuronides of unchanged drug in addition to oxidative N-dealkylated acid. Nebivolol glucuronides (G-UD) comprised a large amount of the EM plasma profile (maximum of 52.9 ng/mL at 2 hours). The plasma of EM subjects also contained glucuronides G-1, G-3, G-6 – G-9, and G-11 and non-conjugated metabolites A-3, A-10, and unchanged nebivolol. In PM plasma G-UD was the largest component (173.0 ng/mL at 4 hours), followed by A-10, and G-8 and unchanged nebivolol.
4. In urine of the EM and PM subjects, 38.4% and 66.5% of the nebivolol dose were recovered. In urine, the major products were conjugated metabolites, N-dealkylated oxidative and hydroxylated conjugates, and small amount of unconjugated metabolites. In EM's urine glucuronide conjugates of unchanged drug or hydroxylated and N-dealkylated metabolites were found. In PM's urine 34.18% of the administered dose were glucuronides of unchanged nebivolol. Minor amounts of conjugates of monohydroxy-nebivolol and non-conjugated metabolites were also present.
5. The fraction of the nebivolol dose recovered in feces of EM and PM subjects was 43.6% and 13.1%, respectively. All metabolites in feces consist of non-conjugated, hydroxylated, or oxidative (i.e., keto) metabolites with transformations occurring on the aliphatic rings and/or aromatic rings. Levels of unchanged drug were much higher in PM than EM subjects. The EM fecal extract contained nine groups of non-conjugated metabolites and unchanged drug. The PM fecal extract contained five minor groups of metabolites and unchanged drug.

4.2.10 An in vitro Study on the Microsomal Metabolism of d- and l-Nebivolol in Human Liver Microsomes (NEBI- 0157)

DRUG STUDIED: **Nebivolol HCL, Janssen Pharmaceuticals Inc.**
SRRR –isomer, Lot # 546730/1, —— purity
RSSS-isomer, Lot# 546731/1, —— purity
Manufacture Date: 8/29/2001
INVESTIGATORS: Andrew Parkinson, Ph. D.

STUDY SITE:

OBJECTIVES:
To evaluate and compare the in vitro metabolism of d- and l-nebivolol with the aim of identifying the human cytochrome P450 (CYP) enzymes involved in their metabolism.

METHODS:
Sample preparation for metabolite identification

Effect of time, protein and substrate concentration
Performed separately, d- and l-nebivolol (0.1, 1 and 10 μ M) were incubated with a single concentration of human liver microsomes (0.25 mg protein/incubation) for 0, 7.5, 15, 30, 45 and 60 min in the presence of NADPH- generating system. Additionally, d- and l-nebivolol (0.1, 1 and 10 μM) were incubated with three concentrations of human liver microsomes (0.125, 0.25 and 0.50 mg protein/incubation) for 15 min. The procedure was the same as that described above. In order to determine Km and Vmax for the metabolism of d- and l-nebivolol by human liver microsomes, 13 concentrations of d- and l-nebivolol (0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.25, 1.6, 2, 4, 7 and 10 μ M) were incubated separately in triplicate with human liver microsomes (0.25 mg protein/incubation) for 15 min. The procedure was the same as that described above.

Identification of human CYP enzymes involved in the metabolism of d- and l-nebivolol to the major metabolite, RT13 (phenotyping) involved three types of analysis:

1. Analysis of the sample- to- sample variation in RT13 formation by a bank of human liver microsomes followed by an analysis of correlations with the sample- to- sample variation in the

activity of the major CYP enzymes expressed in human liver microsomes (namely CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5 and CYPA9/11).
2. Analysis of RT13 formation by human liver microsomes in the presence of monoclonal antibodies that inhibit specific CYP enzymes.
3. Analysis of RT13 formation by recombinant human CYP enzymes.

Human liver microsomes (0.25 mg protein/incubation, n = 16) were incubated with d- or l-nebivolol (1 μM) for 15 min to determine the sample- to sample variation in activity of several CYP enzymes (namely CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5 and CYP4A11). Differences in the rate of formation of metabolite RT13 from d- and l-nebivolol were compared with the sample- to sample variation in the following activities:

CYP1A2	7- Ethoxyresorufin O- dealkylation
CYP2A6	Coumarin 7- hydroxylation
CYP2B6	S- Mephenytoin N- demethylation
CYP2C8	Paclitaxel 6a- hydroxylation
CYP2C9	Diclofenac 4 ′ - hydroxylation
CYP2C19	S- Mephenytoin 4 ′ - hydroxylation
CYP2D6	Dextromethorphan O- demethylation
CYP2E1	Chlorzoxazone 6- hydroxylation
CYP3A4/5	Testosterone 6ß- hydroxylation
CYP4A11	Lauric acid 12- hydroxylation

Antibody inhibition

Human liver microsomes (pool of 16, 0.25 mg protein/mL) were pre-incubated with inhibitory monoclonal antibodies against CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4/5 (12.5, 15, 25, 25, 25, 25, 125, 25 and 125 μ g protein/mL) at room temperature for 15 minutes.

Recombinant human P450 enzymes

Recombinant human CYP enzymes (rCYP enzymes), namely rCYP1A1, rCYP1A2, rCYP2A6, rCYP2B6, rCYP2C8, rCYP2C9, rCYP2C18, rCYP2C19, rCYP2D6, rCYP2E1, rCYP3A4, rCYP3A5, and rCYP4A11 were incubated for 15 min in a 0.5- mL incubation volume with 1 μM d- or l-nebivolol. Incubation conditions, procedures for the HPLC sample preparation and injection volume were the same as that described above.

Data analysis

Data were processed with the spreadsheet computer program Microsoft Excel (Version 8.0, Microsoft Corp., Seattle, WA). Linear regression analysis was performed with proportional

weighting using a computer software program, GraFit (Version 4, Erithacus Software Limited, London, UK). For determination of kinetic constants, the data were analyzed by Lineweaver Burk and/or Eadie- Hofstee plots (nonlinear regression with explicit weighting), and the kinetic constants were estimated using GraFit. Correlation analysis was performed with the computer software SigmaStat Statistical Analysis System (Version 2.0, SPSS Inc., Chicago, IL).

ASSAY:

d- and l-nebivolol and their metabolites were resolved and quantified

Table 72: Assay Characteristics for d- and l-Nebivolol

Parameter	Measure		Reviewer Comment
Linearity			Satisfactory
Precision (CV %)	d-nebivolol	l-nebivolol	Satisfactory
Accuracy Between day	d-nebivolol between	l-nebivolol between -	Satisfactory
LLOQ			Satisfactory
Specificity			Satisfactory

Chromatograms were shown.

A HPLC with LC/MS/MS detection was used for detection of the isolated metabolites from the incubation mixture.

RESULTS:

In the presence of NADPH, d- and l-nebivolol were both metabolized in vitro by human liver microsomes to eight possible metabolites. In both cases, the major metabolite was RT13, which was identified as 4- hydroxynebivolol. The reaction phenotyping part of this study focused on the formation of RT13. The seven other minor metabolites were named RT7, RT8, RT10, RT11, RT12, RT14 and RT15 based on their order of elution (approximate retention time of 7.0, 8.3, 9.9, 10.8, 11.5, 14.0 and 14.5 min, respectively), with RT7 being the most polar metabolite and RT15 being the least polar metabolite.

RT13 formation was proportional to incubation time and protein concentration at all substrate concentrations examined (0.1, 1 and 10 μ M) for both d- and l-nebivolol. The seven minor metabolites were proportional to incubation time and protein concentration at 1 and 10 μ M with a few exceptions (e. g., RT15 formation from d-nebivolol after 45 min), but not at 0.1 μ M.

Table 1 and 2 show that in the presence of NADPH, at (d)- or (l)- nebivolol concentrations of 1 μM) and human liver microsomes of 0.25 mg, the percent loss of (d)- and (l)- nebivolol was 11, 25 and 35%, and 14, 28 and 53% at 7.5-, 15- and 30- min incubations. Mass balance for those samples was 99, 92 and 90% for (d)- nebivolol and 94, 87 and 69% for (l)- nebivolol.

The sponsor determined that more substrate was consumed than the sum of all metabolites. That means that the measurements with the fluorescent detector could be implausible for this assay if the fluorescence properties of the metabolites differed from those of the parent molecule and/or that one or more metabolites were not detected with the fluorescence detector. These results suggested that the initial rate conditions (i. e., conditions under which metabolite formation or substrate disappearance is directly proportional to incubation time and protein concentration and the metabolism of the substrate is not > 20%) achieved by incubating (d)- and (l)- nebivolol with human liver microsomes at 0.25 mg protein/incubation for 15 min.

Table 73: Loss of Substrate and Mass Balance for d-Nebivolol

Concentration of (*d*)-nebivolol	Protein concentration (mg/inc.)	Incubation time (min)	Percent loss of substrate	Percent mass balance
0.1 µM	0.25	0	NA	100%
0.1 µM	0.25	7.5	21.8%	100.8%
0.1 µM	0.25	15	39.8%	87.0%
0.1 µM	0.25	30	62.7%	89.4%
0.1 µM	0.125	15	30.9%	90.5%
0.1 µM	0.25	15	41.0%	87.5%
0.1 µM	0.50	15	48.9%	83.2%
1µM	0.25	0	NA	100%
1 µM	0.25	7.5	10.8%	98.6%
1µM	0.25	15	25.3%	91.7%
1 µM	0.25	30	35.3%	89.9%
1 µM, Zero-Protein	0	15	-6.0%	106.1%
1 µM	0.125	15	14.0%	98.8%
1 µM	0.25	15	32.8%	83.7%
1 µM	0.50	15	37.7%	83.8%
1 µM, Zero-NADPH	0.25	0	NA	100%
1 µM, Zero-NADPH	0.25	15	9.6%	90.6%
10 µM	0.25	0	NA	100%
10 µM	0.25	7.5	4.7%	98.9%
10 µM	0.25	15	10.0%	97.8%
10 µM	0.25	30	18.4%	96.7%
10 µM	0.125	15	4.4%	100.9%
10 µM	0.25	15	10.8%	97.0%
10 µM	0.50	15	21.4%	89.1%

Table 74: Loss of Substrate and Mass Balance for l-Nebivolol

Concentration of (*l*)-nebivolol	Protein concentration (mg/inc.)	Incubation time (min)	Percent Loss of Substrate	Percent Mass Balance
0.1 µM	0.25	0	NA	100%
0.1 µM	0.25	7.5	29.4%	90.2%
0.1 µM	0.25	15	50.7%	80.4%
0.1 µM	0.25	30	72.6%	71.9%
0.1 µM	0.125	15	40.1%	85.6%
0.1 µM	0.25	15	49.8%	81.1%
0.1 µM	0.50	15	56.6%	78.5%
1 µM	0.25	0	NA	100%
1 µM	0.25	7.5	13.7%	94.1%
1 µM	0.25	15	27.7%	86.7%
1 µM	0.25	30	53.3%	68.9%
1 µM, Zero-Protein	0	15	-8.6%	108.6%
1 µM	0.125	15	14.2%	96.3%
1 µM	0.25	15	38.4%	75.7%
1 µM	0.50	15	49.4%	69.1%
1 µM, Zero-NADPH	0.25	0	NA	100%
1 µM, Zero-NADPH	0.25	15	1.7%	98.6%
10 µM	0.25	0	NA	100%
10 µM	0.25	7.5	4.1%	98.8%
10 µM	0.25	15	10.4%	95.8%
10 µM	0.25	30	19.3%	92.5%
10 µM	0.125	15	4.9%	99.3%
10 µM	0.25	15	10.0%	95.9%
10 µM	0.50	15	22.6%	85.2%

Mass-balance and substrate loss is shown in Table 75 and Table 76.
Table 3.

Table 75: Effect of Time, Protein and Substrate Concentration (d-Nebivolol)

Concentration of (*d*)-nebivolol	Protein concentration (mg/inc.)	Incubation time (min)	Percent Loss of Substrate	Percent Mass Balance
0.1 μM	0.25	0	NA	100%
	0.25	7.5	34.7%	79.1%
	0.25	15	57.2%	66.0%
	0.25	30	81.4%	44.3%
	0.25	45	89.5%	44.2%
	0.25	60	92.8%	33.5%
	0	15	2.3%	97.8%
	0.125	15	48.4%	73.4%
	0.25	15	59.2%	67.7%
	0.50	15	66.1%	62.9%
1 μM	0.25	0	NA	100%
	0.25	7.5	15.4%	92.0%
	0.25	15	27.7%	87.0%
	0.25	30	45.9%	80.9%
	0.25	45	59.0%	73.7%
	0.25	60	68.6%	68.7%
	0	15	4.8%	95.2%
	0.125	15	18.0%	92.2%
	0.25	15	29.4%	86.8%
	0.50	15	43.4%	78.2%
10 μM	0.25	0	NA	100%
	0.25	7.5	3.6%	99.8%
	0.25	15	7.3%	99.6%
	0.25	30	14.0%	98.7%
	0.25	45	23.4%	94.1%
	0.25	60	29.4%	92.5%
	0	15	-3.1%	103.1%
	0.125	15	3.2%	101.4%
	0.25	15	4.3%	102.5%
	0.50	15	16.9%	92.3%

Table 76: Effect of Time, Protein and Substrate Concentration (l-Nebivolol)

Concentration of (l)-nebivolol	Protein concentration (mg/inc.)	Incubation time (min)	Percent Loss of Substrate	Percent Mass Balance
0.1 µM	0.25	0	NA	100%
	0.25	7.5	46.2%	65.2%
	0.25	15	68.5%	47.8%
	0.25	30	85.3%	33.4%
	0.25	45	93.2%	24.6%
	0.25	60	95.0%	19.9%
	0	15	2.3%	97.7%
	0.125	15	60.7%	53.9%
	0.25	15	70.3%	46.9%
	0.50	15	76.1%	40.4%
1 µM	0.25	0	NA	100%
	0.25	7.5	21.0%	86.6%
	0.25	15	34.7%	79.2%
	0.25	30	54.8%	68.1%
	0.25	45	69.8%	57.3%
	0.25	60	77.9%	52.3%
	0	15	8.6%	91.4%
	0.125	15	21.6%	90.6%
	0.25	15	35.3%	83.7%
	0.50	15	54.1%	67.4%
10 µM	0.25	0	NA	100%
	0.25	7.5	2.2%	101.2%
	0.25	15	9.0%	97.4%
	0.25	30	18.4%	93.3%
	0.25	45	22.6%	93.6%
	0.25	60	28.6%	91.0%
	0	15	0.3%	99.7%
	0.125	15	4.3%	100.4%
	0.25	15	10.2%	96.0%
	0.50	15	17.1%	91.5%

The kinetic constants (Km and Vmax) were determined for the major metabolite (RT13) by incubating human liver microsomes (0.50 mg protein/mL) for 15 min with 13 concentrations of d- or l-nebivolol (0.4, 0.5, 0.6, 0.7, 0.8, 0.9, 1, 1.25, 1.6, 2, 4, 7 and 10 µ M). Example of Lineweaver plot for d-nebivolol is shown below (Figure 41).

Since the regression line did not describe the relationship well, the sponsor proposed that at least two enzymes are involved in the formation of R13 metabolite differing in affinity. However, using the data from this experiment, only parameters for the high affinity enzyme were calculated. The parameters calculated for the low affinity enzyme were of unreasonable values.

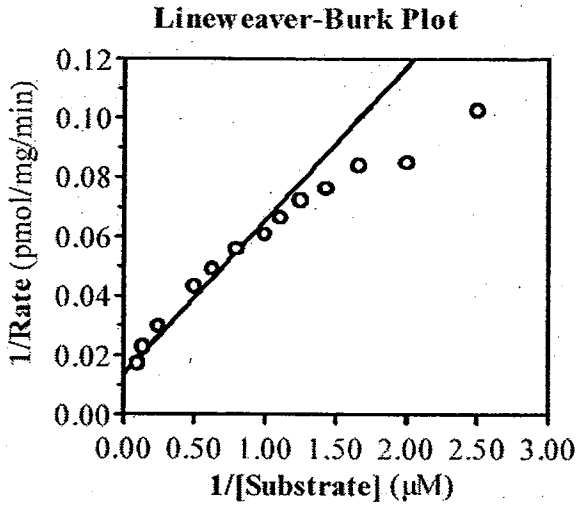

Figure 41: Lineweaver-Burk plot

The apparent Km, Vmax and the in vitro intrinsic clearance, Vmax/Km, of RT13 for the metabolism of d- and l-nebivolol are summarized below.

Table 77: Kinetic Constants for R-13 Formation

Substrate	Enzyme	Km (μM)	Vmax (pmol/mg/min)	Vmax/Km (μL/mg/min)
(*d*)-nebivolol	High Affinity	0.84	30	36
	Low Affinity	ND	ND	ND
(*l*)-nebivolol	High Affinity	3.8	48	13
	Low Affinity	ND	ND	ND

Although the in vitro experiment is easy to perform, the sponsor did not attempt to characterize the kinetic parameters more precisely.

Formation of RT13 correlated strongly with CYP2D6 (dextromethorphan O-demethylase) activity, with a correlation coefficient (r) of 0.932 for d-nebivolol and 0.925 for l-nebivolol. No other CYP activity showed a significant correlation with RT13 formation.

Inhibitory monoclonal antibodies against CYP enzymes (CYP1A2, CYP2A6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1 and CYP3A4/5) were incubated with a pool of human liver microsomes to examine their effects on the metabolism of d- and l-nebivolol (1 μ M). Monoclonal antibody against CYP2D6 inhibited the conversion of d- and l-nebivolol to RT13 by 73% and 75%, respectively. Monoclonal antibody against CYP3A4/5 inhibited the conversion of d- and l-nebivolol to RT13 by 16% and 20%, respectively. No other antibody affected the formation of RT13 by more than 10%.

Each of d- and l-nebivolol (1 μ M) were incubated separately with recombinant human enzymes (CYP1A1, CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C18, CYP2C19, CYP2D6, CYP2E1, CYP3A4, CYP3A5 and CYP4A11) at 25 pmol/incubation. Formation of RT13 from both d- and l-nebivolol was observed in incubations with rCYP1A1, rCYP2C19, rCYP2D6 and

rCYP3A4. For both d- and l-nebivolol, the percent loss of substrate with rCYP1A1 and rCYP2C19 was minimal while the percent loss of substrate with rCYP2D6 and rCYP3A4 was 96.5%, 13.4%, 98.6% and 9.1%, respectively. These results suggest that for both d- and l-nebivolol, rCYP2D6 was a significant contributor to the formation of RT13 as affirmed by the results of the correlation analysis.

COMMENTS:

1. The sponsor determined that d- and l-nebivolol were metabolized by human liver microsomes to one major metabolite, RT13 (4- hydroxynebivolol), and seven minor metabolites, RT7, RT8, RT10, RT11, RT12, RT14 and RT15. CYP2D6 was the major enzyme responsible for converting both d- and l-nebivolol to its major metabolite, and CYP3A4 contributes to the formation of RT13 from both enantiomers.

2. The sponsor proposed that the major metabolite of d- and l-Nebivolol is 4-hydroxynebivolol (RT13). The sponsor proposed the structures of the metabolites based on the correspondence of the retention times of the eluted substances with the mass-ions of 406 (nebivolol), 422 (possibly hydroxylated nebivolol) and 438 (possibly dihydroxylated nebivolol). Exact determination of the chemical structure of the eluted compounds was not performed. The position of the hydroxy group was proposed based on the retention times which were similar to the monohydroxynebivolol identified in the mass balance study. These explanations can be considered only as speculations.

3. Recombinant CYP2D6 also converted d- and l-nebivolol to RT13. During this reaction, the loss of d- and l-nebivolol was not accounted for by the formation of RT13. The sponsor speculated that both d- and l-nebivolol were extensively metabolized by CYP2D6, and that RT13 maybe further metabolized to a non-fluorescent metabolite(s). Although the study proved that the major metabolic pathway is associated with CYP2D6, and the minor pathway – with CYP3A4, the quantitative description of this process should be considered as preliminary results.

4. For the assay of the parent drug and metabolites the sponsor used the HPLC method with fluorescent detection. There is no information available regarding the fluorescent properties of the possible metabolites of nebivolol. Moreover, the results of the mass-balance for the substrate indicate that the recovery was as low as 20% (at low, 0.1 mcM nebivolol concentration). That means that there were up to 80% of the metabolites that could not be measured using the fluorescent detection.

5. Calculation of the Km and Vmax values using the linear regression of the derived data could lead to false results. There is no limitation for the amount of data point, which could be obtained, in the in vitro study. It is plausible to describe the data using the Michaelis-Menthen equation in the explicit form. That would give a direct estimation of the parameters for the high- and low-affinity enzymes. The calculations based on the regression performed with Excel are very approximate and do not have any physiologic value.

4.2.11 An in vitro Evaluation of Nebivolol as an Inhibitor of Human Cytochrome P450 Enzymes (NEBI-158)

Authors: **Kennedy B,**
Testing Facility:

DRUG STUDIED: **14C-Nebivolol Hydrochloride, 15mg**
Mylan Pharmaceuticals Inc. Lot No.: X1020: 22A

OBJECTIVES:
To evaluate the ability of nebivolol to inhibit the major cytochrome P450 enzymes in human liver microsomes in vitro (namely CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5 [using two different substrates] and CYP4A9/11), with the aim of ascertaining the potential of nebivolol to inhibit the metabolism of concomitantly administered drugs
To measure the inhibitory constant (Ki value) of the compound for the reversible metabolism-"independent" inhibition of each human P450 enzyme examined.
To determine whether or not nebivolol is a reversible or irreversible metabolism- dependent inhibitor of the same enzymes.

METHODS
Human liver microsomes from a pool of nine individuals were incubated with marker substrates at concentrations approximately equal to 1/2, 1, 2 and 4 times the Km for each marker. Marker substrate concentrations were reduced for CYP2B6 (1/4, 1/2, 2/3, and 1 times Km), CYP2C8 (1/3, 2/3, 1, and 4/3 times Km) and CYP3A4/5 (testosterone 6ß- hydroxylase; 1/3, 1/2, 1, and 2 times Km. All incubations were performed in the presence or absence of nebivolol at target concentrations ranging from 0.1 to 50 μ M. In addition, nebivolol was evaluated for its ability to function as a metabolism- dependent (mechanism- based) reversible or irreversible inhibitor, in which case, nebivolol was pre- incubated with human liver microsomes and NADPH for 15 min to allow for the generation of metabolites that could inhibit cytochrome P450. Whenever possible, known metabolism- independent or metabolism- dependent inhibitors of P450 enzymes were included as positive controls.

STATISTICS
Data were processed with a spreadsheet computer program Microsoft Excel. When inhibition of P450 enzymes was observed, Ki values were calculated with a GraFit computer software program (version 4.0, Erithacus Software Limited, London, UK). The data were then plotted on an Eadie- Hofstee plot. For determination of Ki values, the entire data set was fitted to the Michaelis-Menten equations for competitive, noncompetitive, uncompetitive and mixed inhibition by nonlinear regression analysis with simple weighting. The goodness evaluated by a lower reduced chi- squared value. A relatively high standard deviation associated with Ki values suggests that the nonlinear regression did not fit the data very well, and a visual inspection of the Eadie- Hofstee plot may be necessary to confirm the nature of inhibition. When extrapolation or interpolation of Ki values beyond the concentration range studied was required, these values are provided as estimates only.

RESULTS:

Evaluation of nebivolol as a metabolism- independent inhibitor: Determination of Ki values

Nebivolol caused concentration dependent inhibition of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP3A4/5 (midazolam 1´-hydroxylase and testosterone 6ß-hydroxylase) and CYP4A9/11. The inhibition of CYP2A6, CYP2B6, CYP2C19 and CYP2D6 appeared to be competitive in nature with Ki values of 49, 92, 130 and 0.37 μ M, respectively.

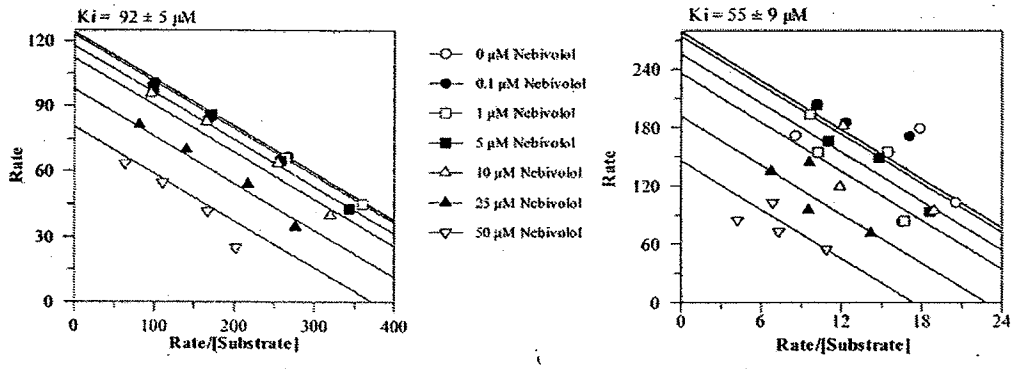

Figure 42: Metabolism- independent inhibition of CYP1A2 and CYP2C8 by nebivolol

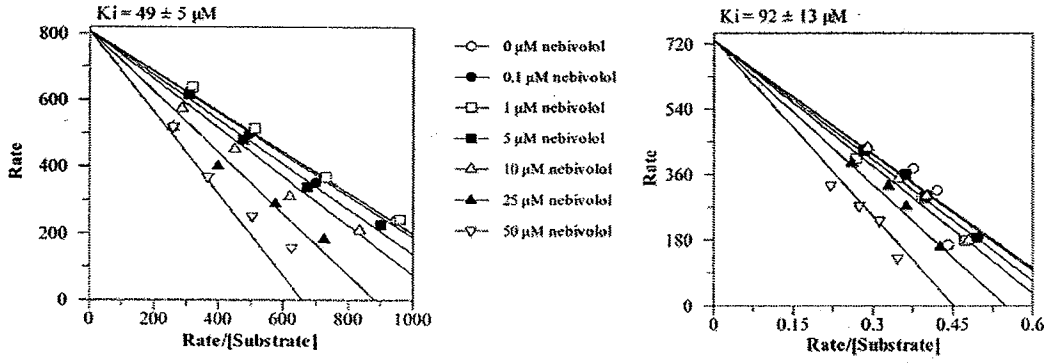

Figure 43: Metabolism- independent inhibition of CYP 2A6 and CYP2B6 by nebivolol

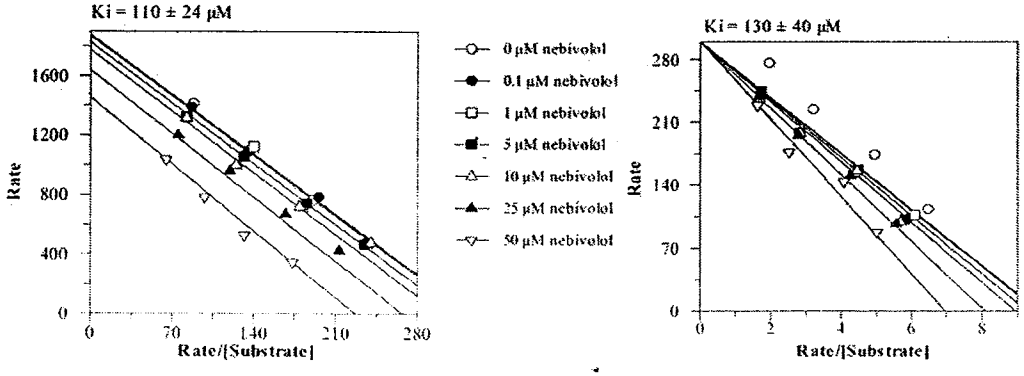

Figure 44: Metabolism- independent inhibition of CYP2C9 and CYP2C19 by nebivolol

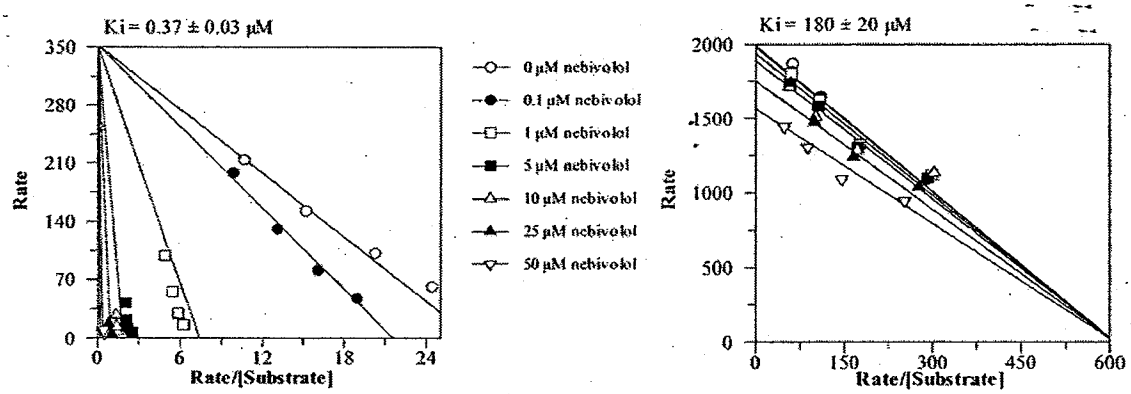

Figure 45: Metabolism- independent inhibition of CYP2D6 and CYP4A9/11by nebivolol

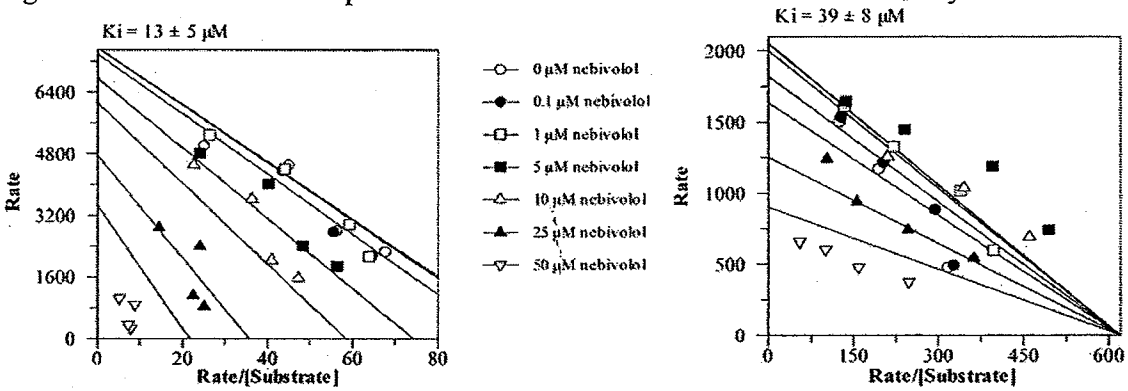

Figure 46: Metabolism- independent inhibition of CYP3A4/5 (testosterone 6ß-hydroxylase, left panel) and (midazolam 1′-hydroxylase, right panel) by nebivolol

The inhibition of CYP1A2 and CYP2C8 appeared to be noncompetitive in nature with respective Ki values of 92 and 55 µ M, respectively, while the inhibition of CYP3A4/5 (midazolam 1′-hydroxylase) and CYP4A9/11 appeared to be uncompetitive in nature with Ki values of 39 and 180 µ M, respectively. Additionally, nebivolol appears to be a mixed (competitive-noncompetitive) inhibitor of CYP2C9 and CYP3A4/5 (testosterone 6ß- hydroxylase) with Ki values of 110 and 13 µ M, respectively. Finally, nebivolol has little or no capacity to function as a metabolism independent inhibitor of CYP2E1.

Based on the information on the in vitro inhibitory potential of nebivolol, the most significant effect in vivo would be a predicted inhibition of CYP2D6- mediated processes. The degree to which CYP2D6 is inhibited in vivo may exceed the in vitro estimate if nebivolol attains hepatic levels that greatly exceed blood levels. Because the fractional inhibition of CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5 and CYP4A9/11 are predicted to be relatively low, nebivolol would not be expected to inhibit the clearance of concomitantly administered drugs that are metabolized by these enzymes.

Evaluation of nebivolol as a reversible metabolism dependent inhibitor

Nebivolol did not cause reversible metabolism- dependent inhibition of any of the P450 enzymes examined (Figure 47).

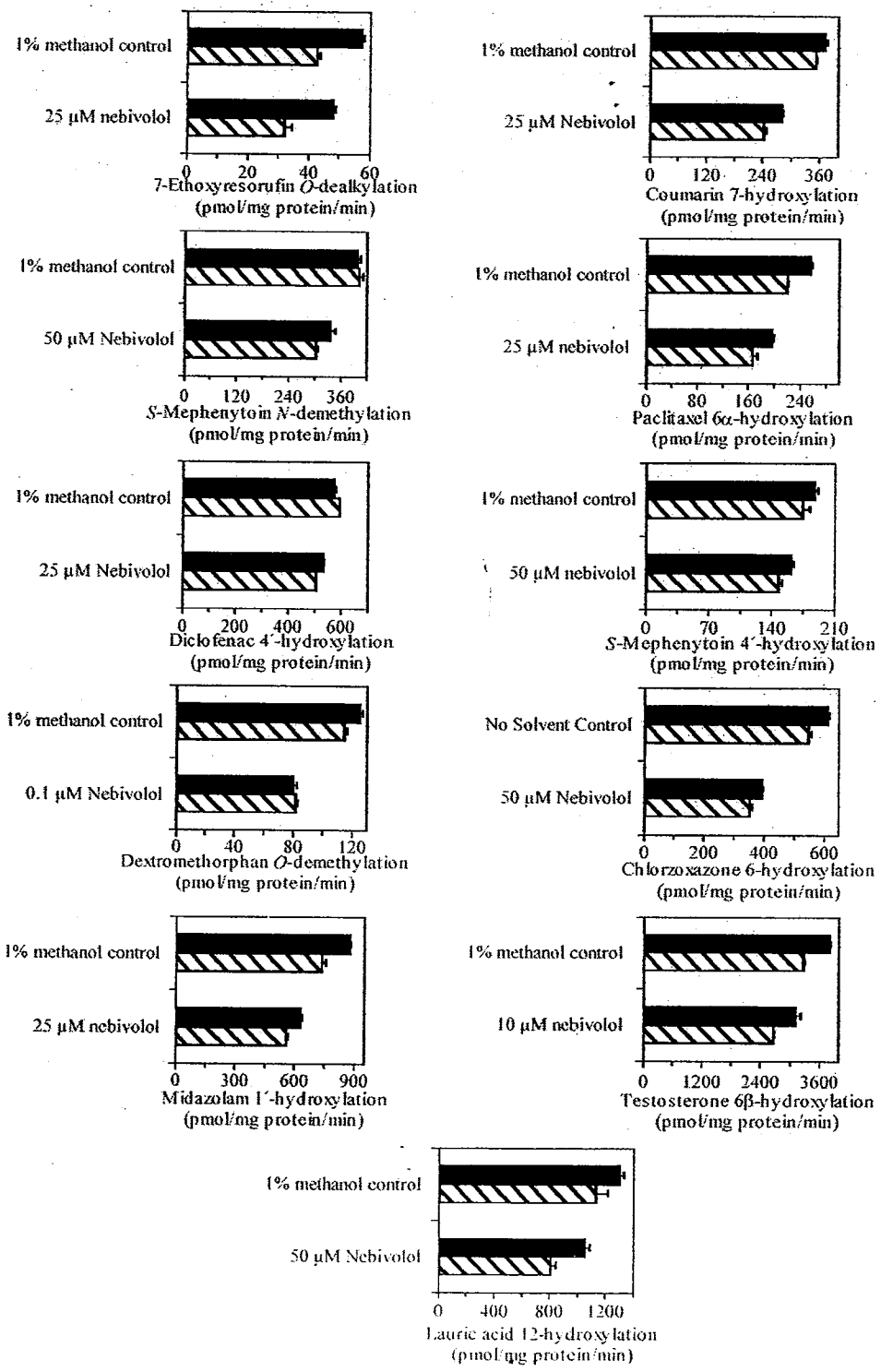

Figure 47: Metabolism- dependent reversible inhibition of human P450 enzymes by nebivolol

Evaluation of nebivolol as an irreversible metabolism-dependent inhibitor

Nebivolol did not cause irreversible metabolism- dependent inhibition of any of the P450 enzymes examined (Figure 48).

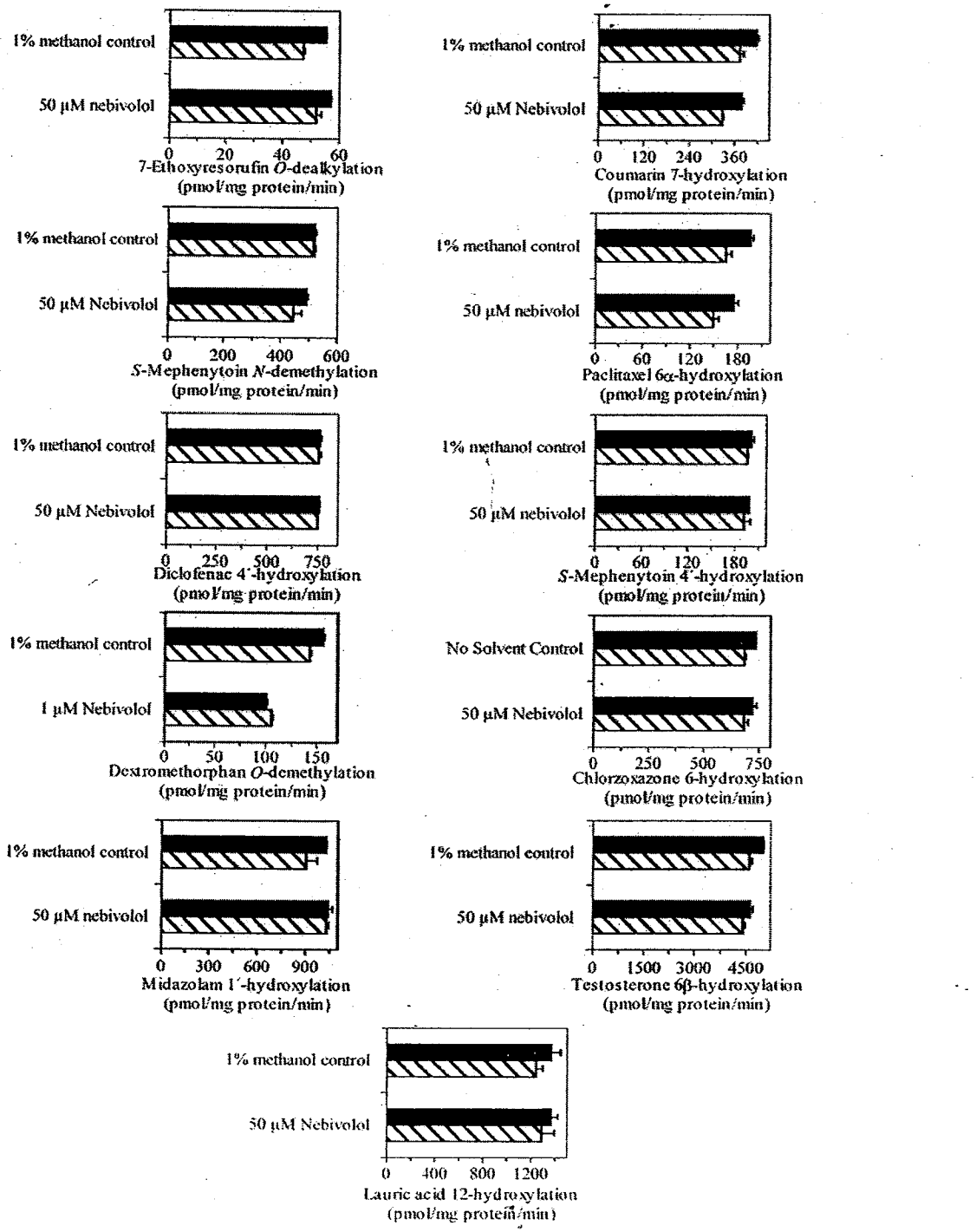

Figure 48: Metabolism- dependent irreversible inhibition of human P450 enzymes by nebivolol

The calculated Ki values are summarized in Table 78.

Table 78: CYP450 Ki Values

Enzyme	P450 Activity	Metabolism-independent inhibition		
		Ki [a] (μM)	*i* [c]	[I]/Ki [d]
CYP1A2	7-Ethoxyresorufin *O*-dealkylase	92 ± 5^{NC}	0.02%	0.00018
CYP2A6	Coumarin 7-hydroxylase	49 ± 5^{CI}	0.03%	0.00035
CYP2B6	*S*-Mephenytoin *N*-demethylase	92 ± 13^{CI}	0.02%	0.00018
CYP2C8	Paclitaxel 6α-hydroxylase	55 ± 9^{NC}	0.03%	0.00031
CYP2C9	Diclofenac 4'-hydroxylase	110 ± 24^{MI}	0.02%	0.00015
CYP2C19	*S*-Mephenytoin 4'-hydroxylase	130 ± 40^{CI}	0.01%	0.00013
CYP2D6	Dextromethorphan *O*-demethylase	0.37 ± 0.03^{CI}	4.39%	0.04595
CYP2E1	Chlorzoxazone 6-hydroxylase	>300 [b]	NA	NA
CYP3A4/5	Midazolam 1'-hydroxylase	39 ± 8^{UC}	0.04%	0.00044
CYP3A4/5	Testosterone 6β-hydroxylase	13 ± 5^{MI}	0.13%	0.00131
CYP4A9/11	Lauric acid 12-hydroxylase	180 ± 20^{UC}	0.01%	0.00009

COMMENTS:-

1. The sponsor reported that in vitro nebivolol appears to be a competitive inhibitor of CYP2A6, CYP2B6, CYP2C19, and CYP2D6 with Ki values of 49, 92, 130 and 0.37 μ M, respectively;
2. Nebivolol appears to be a noncompetitive inhibitor of CYP1A2 and CYP2C8 with Ki values of 92 and 55 μ M, respectively;
3. Nebivolol appears to be a uncompetitive inhibitor of CYP3A4/5 (as measured by midazolam 1 ′ - hydroxylation) and CYP4A9/11 with Ki values of 39 and 180 μ M, respectively;
4. Nebivolol appears to be a mixed (competitive/noncompetitive) inhibitor of CYP2C9 and CYP3A4/5 (as measured by testosterone 6ß- hydroxylation) with Ki values of 110 and 13 μ M, respectively.
5. The rank order of Ki values for the inhibition of the enzymes listed above is as follows: CYP2D6 < CYP3A4/5 (testosterone 6ß- hydroxylase) < CYP3A4/5 (midazolam 1 ′ - hydroxylase) < CYP2A6 < CYP2C8 < CYP1A2 ~ CYP2B6 < CYP2C9 < CYP2C19 < CYP4A9/11. 5) nebivolol does not have the capacity to function as a direct- acting (metabolism- independent) reversible inhibitor of CYP2E1.
6. Nebivolol has little or no capacity to function as a reversible or irreversible metabolism- dependent inhibitor of any of the P450 enzymes examined.
7. The sponsor reasonably predicted that the inhibition of CYP2D6- mediated processes is the most significant effect in vivo. Nebivolol is not expected to inhibit the clearance of concomitantly administered drugs that are metabolized by the following enzymes: CYP1A2, CYP2A6, CYP2B6, CYP2C8, CYP2C9, CYP2C19, CYP2D6, CYP2E1, CYP3A4/5 and CYP4A9/11.

4.2.12 A Randomized, Parallel Group Safety Evaluation of Electrocardiographic Intervals And Blood Pressure in Normal Healthy Volunteers after Nebivolol, Atenolol, Moxifloxacin, or Placebo Administration after Single and Repeated Doses (NEB122)

INVESTIGATIONAL PRODUCT:	**Nebivolol hydrochloride**
DEVELOPMENT PHASE:	**PHASE 1**
STUDY DATES:	**June 2, 2003 - July 28, 2003**
PRINCIPAL INVESTIGATOR:	**L. A. Galitz, M. D**

OBJECTIVES:

To document the effects of nebivolol on the electrocardiographic intervals of normal healthy volunteers administered nebivolol, 20 and 40 mg, for 7 days. The working hypothesis was that 20 or 40 mg of nebivolol would not prolong corrected QT intervals measured during peak nebivolol concentrations (i. e., 2 hours after dosing) on Day 7.

STUDY DESIGN:

This was a randomized, placebo- and active-controlled, parallel group study of nebivolol in healthy subjects. The study was not blinded, although personnel at the central laboratory for ECG measurement and interpretation were blinded to time and treatment.

There were an initial screening (Visit 1) and 7- day treatment period (Visit 2). The subjects were stratified by sex and randomized equally in a 1: 1: 1: 1 ratio to one of four treatment groups: nebivolol, atenolol, moxifloxacin, or placebo. The poor metabolizers of CYP2D6 were preferentially randomized equally to either nebivolol or atenolol treatment groups.

Table 79 shows the drug schedule and doses for the four treatments groups.

Table 79: Treatment Schedule

Group	Drug		Treatment Schedule
1	nebivolol	active drug	20 mg qd x 3 days and 40 mg qd x 4 days or 20 mg qd x 7 days
2	placebo	negative control	1 tablet qd x 7 days
3	atenolol	active control	100 mg qd x 3 days and 200 mg qd x 4 days or 100 mg qd x 7 days
4	moxifloxacin	positive control	400 mg qd x 7 days

Subjects received study medication once daily in the morning for 7 days. Continuous 24-hour 12-lead ECG records were obtained on Days 0, 1, 4, and 7 of dosing. A single 12-lead tracing was pulled by the central laboratory for interval measurement from each continuous ECG record at the same times that blood samples were collected for plasma drug analysis — immediately before and 0.5, 1, 1.5, 2, 2.5, 3, 4, 6, 10, 12, 14, 16, 18, and 24 hours after dosing. At least three consecutive ECG intervals for each tracing were measured by the central laboratory. In addition to continuous ECGs, standard office 12- lead ECG records were obtained every 12 hours until discharge as safety assessments.

ASSAY:

Concentrations of nebivolol (d- and l-enantiomers), atenolol and moxifloxacin in human plasma samples collected during the study were determined using a validated liquid chromatography/single mass spectrometry (LC/MS) bioanalytical methods .

The assay for nebivolol utilized three different standard curve ranges. The method for the analysis of d-nebivolol and l-nebivolol in human plasma (heparin) was performed using high performance liquid chromatography with tandem mass spectrometric detection.

Table 80: Assay Characteristics for d- and l-Nebivolol

Parameter	Measure		Reviewer Comment
	Assay for Curve I		
Linearity	0.02ng/mL to 1.5ng/mL		Satisfactory
Precision (CV %)	d-nebivolol ≤ 6.17	l-nebivolol ≤ 6.02	Satisfactory
Accuracy Between day	d-nebivolol between –3.4% and 5.8%	l-nebivolol between -3.18% and 5.41%	Satisfactory
LLOQ	0.02ng/mL		Satisfactory
Specificity			Satisfactory
	Assay for Curve II		
Linearity	0.2ng/mL to 15.0ng/mL		Satisfactory
Precision (CV %)	d-nebivolol ≤ 4.31	l-nebivolol ≤ 6.50	Satisfactory
Accuracy Between day	d-nebivolol between –4.92% and 4.50%	l-nebivolol between -2.11% and 3.21%	Satisfactory
LLOQ	0.02ng/mL		Satisfactory
Specificity			Satisfactory
	Assay for Curve III		
Linearity	0.04ng/mL to 3.0ng/mL		Satisfactory
Precision (CV %)	d-nebivolol ≤ 3.97	l-nebivolol ≤ 2.28	Satisfactory
Accuracy Between day	d-nebivolol between –4.09% and 6.24%	l-nebivolol between -4.68% and 4.91%	Satisfactory
LLOQ	0.02ng/mL		Satisfactory
Specificity			Satisfactory

Chromatograms were shown.

STATISTICAL ANALYSES

An analysis of covariance (ANCOVA) with treatment as the main effect and baseline QTc and gender as the covariates was used to analyze changes in QTc intervals for the average of 15 time points on Day 0 (baseline) and individual subject's time points on Day 7, with the primary objective being an analysis of change from baseline to 2 hours after dosing on Day 7.

QT intervals were corrected for variations in HR using a population correction factor (derived from all Day 0 continuous 12- lead ECG data) and two standard formulas

Bazett's: $QTc\text{-}B = QT/(RR)^{1/2}$ and

Fridericia's: $QTc\text{-}F = QT/(RR)^{1/3}$).

The following comparisons were evaluated:

nebivolol-placebo treatment difference was tested by computing a point estimate and 95% confidence interval (primary assessment),

pairwise comparisons of nebivolol to moxifloxacin and atenolol were performed (secondary assessment), and

moxifloxacin was compared to placebo to ensure the sensitivity of the ECG analyses.

Treatment groups were compared for demographics (age, gender, race, oxidative genotype) and baseline measurements (vital signs, physical examinations, ECGs, seated blood pressures and pulse rates, and clinical laboratory assessments) using a fixed effects analysis of variance (ANOVA) for continuous variables and Pearson's chi-square test for discrete variables. Other continuous variables, including plasma concentrations, were summarized by treatment using descriptive statistics, and categorical variables were tabulated by treatment using the number and percentage of subjects by category.
All statistical analyses were performed using the SAS ® System, Version 8.2 (SAS Institute). No rules for imputing values for missing data were applicable.

RESULTS:
The treatments formulations, lot numbers and manufacturing date are shown in Table 81.

Table 81: Study drug

Drug Product	Lot Numbers
Nebivolol HCl 20 mg tablets Mylan Pharmaceuticals Inc	R1K3674
Atenolol 100 mg tablets (Tenormin®) AstraZeneca Pharmaceuticals	ABB331 and ABB341
Moxifloxacin HCl 400 mg tablets (Avelox®) Bayer Pharmaceuticals Corporation	2500CL3
Placebo tablets Mylan Pharmaceuticals Inc	R1H1956

A total of 285 subjects were enrolled, and 269 subjects completed the study (71, 61, 68, and 69 in the nebivolol, atenolol, moxifloxacin, and placebo treatment groups, respectively). Among all randomized subjects, 65 subjects in the nebivolol group had their daily dose increased from 20 mg to 40 mg and 60 subjects in the atenolol group had their daily dose increased from 100 mg to 200 mg based on Day 4 telemetry results indicating HR> 51.
Genotyping identified 3 subjects (# 069, 141, 260) in the nebivolol treatment group (4.2%) and 3 subjects (# 070, 142, 219) in the atenolol treatment group (4.3%, 3/70) as poor metabolizers.
Summary of the demographic characteristics provided in Table 82.

Table 82: Summary of the demographic characteristics by treatment

Demographic Characteristic	Nebivolol (N = 72)	Atenolol (N = 70)	Moxifloxacin (N = 71)	Placebo (N = 72)	p-value[a]
	n (%)	n (%)	n (%)	n (%)	
Poor Metabolizers	3 (4.2)	3 (4.3)	NA	NA	NA
Gender					
Male	44 (61.1)	35 (50.0)	29 (40.8)	38 (52.8)	0.1121
Female	28 (38.9)	35 (50.0)	42 (59.2)	34 (47.2)	
Ethnic Group					
Caucasian	37 (51.4)	38 (54.3)	37 (52.1)	33 (45.8)	
Hispanic	27 (37.5)	27 (38.6)	29 (40.8)	24 (33.3)	0.1792
Black	8 (11.1)	5 (7.1)	5 (7.0)	15 (20.8)	
Frame Size					
Medium	39 (54.2)	43 (61.4)	49 (69.0)	43 (59.7)	
Large	25 (34.7)	25 (35.7)	20 (28.2)	25 (34.7)	0.2453
Small	8 (11.1)	2 (2.9)	2 (2.8)	4 (5.6)	
Age (yr)					
Mean (SD)	45.0 (13.39)	45.0 (13.38)	45.7 (12.87)	45.3 (13.81)	
Median	45.0	45.5	45.0	46.5	0.9874
(Min-Max)	(18-75)	(18-76)	(19-77)	(18-75)	
Weight (lb)					
Mean (SD)	160.2 (24.05)	158.9 (24.26)	155.0 (21.52)	159.3 (24.45)	
Median	157.5	151.5	154.0	158.0	0.5757
(Min-Max)	(110-214)	(118-209)	(108-205)	(116-216)	
Height (in)					
Mean (SD)	65.4 (3.63)	64.8 (4.28)	64.9 (3.79)	65.1 (3.91)	
Median	65.0	65.0	65.0	65.0	0.7966
(Min-Max)	(57-72)	(54-73)	(59-73)	(57-73)	

All treatment groups were well matched with respect to subject demographic and baseline characteristics.

The data submitted by the sponsor included only d,l-nebivolol plasma concentrations. The individual plasma d,l-nebivolol concentrations for EMs and PMs on Days 1, 4, and 7 are shown in Figure 49. The EM subjects had reached peak plasma levels at about 1 hour post-dose, and PM subjects reached Tmax at about 6 and 3 hours on Day 1 and at 6 hours on Day 7.

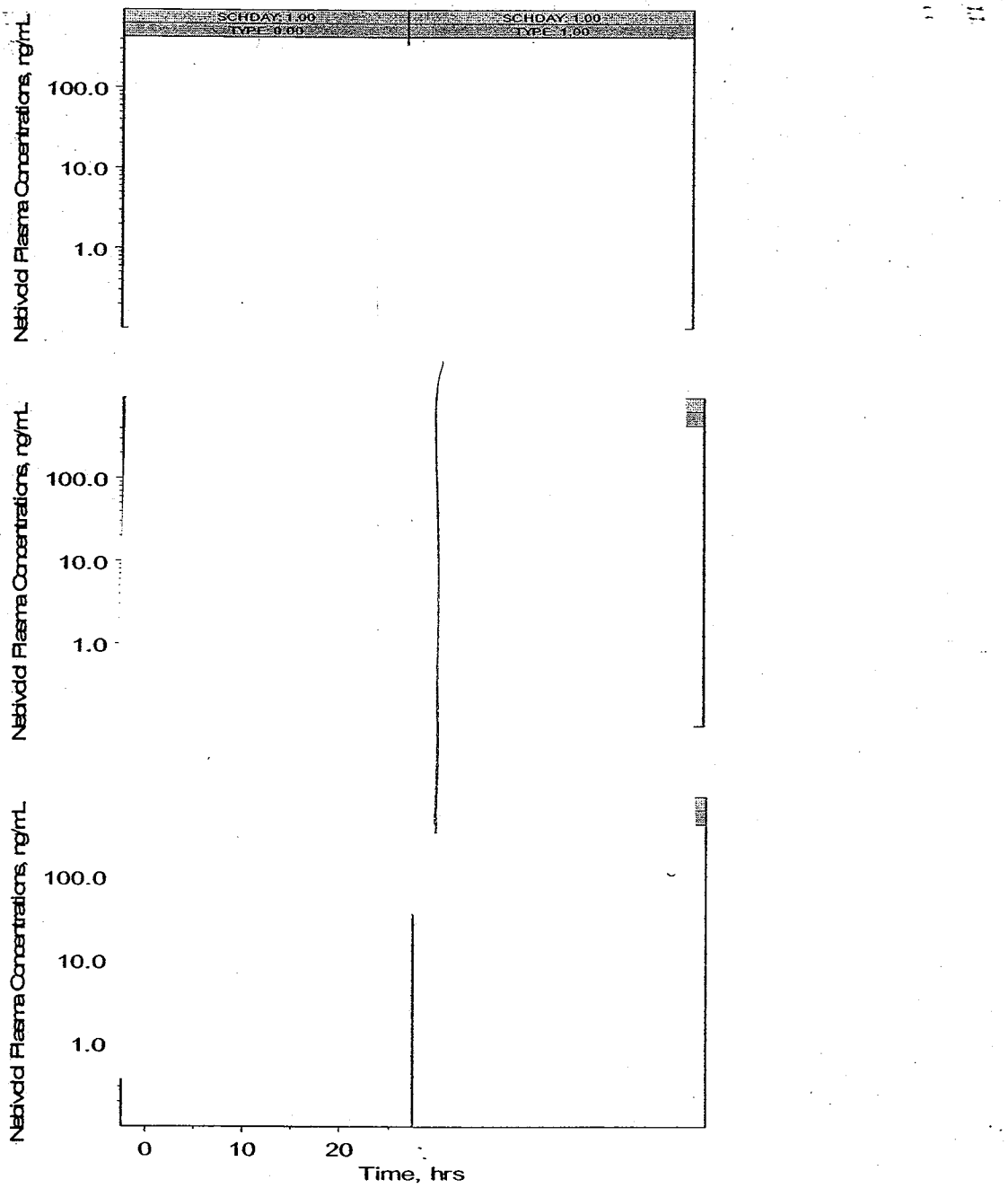

Figure 49: Observed nebivolol plasma concentrations, left panel- EMs, right panel – PMs. Upper panel – Day 1, middle panel – Day 4, lower panel - Day 7

The mean d,l-nebivolol Cmax 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 NEBI-126 a single dose of 20 mg nebivolol led to a Cmax of 4.64 ng/mL for d,l-nebivolol. The 54% higher value for Cmax on Day 1 may be explained by the high inter-patient variability of nebivolol. With respect to Cmax, 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 Cmax from Day 1 (20 mg dose) and Day 4 (40 mg dose after 4 doses of 20 mg) confirms the dose linearity for Cmax. The 30% change in Cmax from Day 4 to Day 7 may be attributed to the drug accumulation.
As a primary endpoint, the sponsor evaluated the change in QTc interval from baseline (Day 0) to 2 hours post-dose on Day 7. The comparisons were made for QTc interval calculated using a population factor, Bazett's and Fredericia's correction methods (Table 83).

Table 83: Comparison of the treatments by QTc interval change from Day 0 to 2 hours post-dose on Day 7

Correction Method, Parameter	Nebivolol vs. Placebo	Moxifloxacin vs. Placebo	Nebivolol vs. Moxifloxacin	Nebivolol vs. Atenolol
Population Factor [a]				
Test–LS (SE)	-5.06 (1.8676)	5.22 (1.9201)	-5.06 (1.8676)	-5.06 (1.8676)
Reference–LS (SE)	-6.21 (1.9009)	-6.21 (1.9009)	5.22 (1.9201)	-4.91 (2.0249)
Difference [b]	1.14	11.43	-10.28	-0.15
95% CI	-4.09 , 6.38	6.09 , 16.76	-15.58 , -4.98	-5.58 , 5.28
P-value [c]	0.6672	0.0000	0.0002	0.9570
Bazett's Formula				
Test–LS (SE)	-20.73 (2.2495)	8.77 (2.3128)	-20.73 (2.2495)	-20.73 (2.2495)
Reference–LS (SE)	-4.86 (2.2908)	-4.86 (2.2908)	8.77 (2.3128)	-21.82 (2.4427)
Difference [b]	-15.86	13.64	-29.50	1.09
95% CI	-22.17 , -9.55	7.21 , 20.06	-35.89 , -23.11	-5.45 , 7.63
P-value [c]	0.0000	0.0000	0.0000	0.7431
Fridericia's Formula				
Test–LS (SE)	-5.70 (1.8725)	5.30 (1.9252)	-5.70 (1.8725)	-5.70 (1.8725)
Reference–LS (SE)	-6.38 (1.9060)	-6.38 (1.9060)	5.30 (1.9252)	-5.40 (2.0303)
Difference [b]	0.68	11.68	-11.00	-0.31
95% CI	-4.57 , 5.93	6.33 , 17.02	-16.32 , -5.68	-5.75 , 5.13
P-value [c]	0.7996	0.0000	0.0001	0.9111

The administration of the positive control, moxifloxacin, increased QTc interval calculated with any correction method, and a 6msec change is detectable in this study. At low heart rates, the corrections with the Fridericia's formula would give less error. In the comparison of nebivolol versus placebo, the mean difference in QTc interval (95% CI) at 2 hours after dosing on Day 7 (peak effect) was 1.14 msec (- 4.09 , 6.38) using the population correction factor and 0.68 msec (- 4.57 , 5.93) using Fridericia's formula. Although the upper bound of the 95% confidence interval was slightly higher than 6 msec for the population corrected data, the small differences (point estimates) in the change from mean baseline QTc intervals between nebivolol and placebo demonstrate the difference between nebivolol and placebo treatments was not statistically significant (p= 0.6672, population and 0.7996 Fridericia's correction).
The treatment with nebivolol and atenolol (active control) showed similar results, with mean reductions in QTc of approximately 5 msec for both treatments at the time of peak effect.
The sponsor made pairwise comparisons of the changes in QTc interval from baseline to all time points on Day 7, Figure 50.

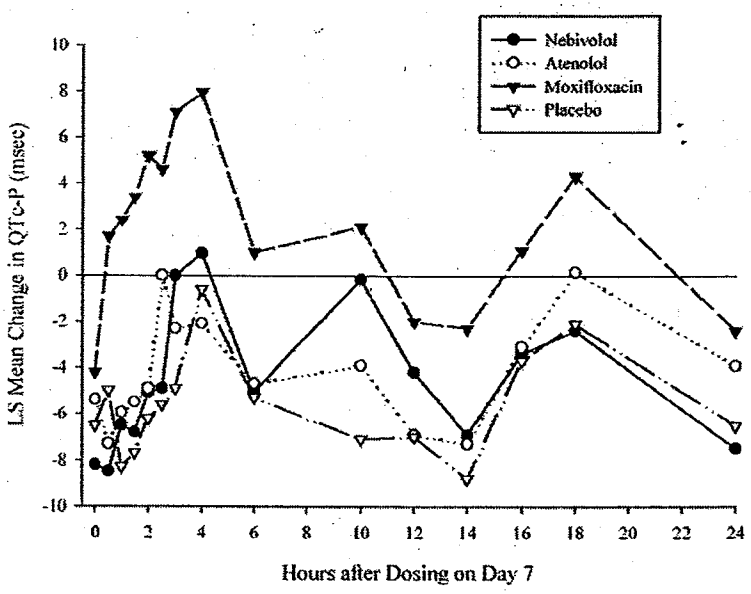

Figure 50: Least Squares (LS) Mean Change from Baseline in QTc- P Intervals on Day 7.

In this plot, the mean changes of QTc interval were above 0 at 4 hours post dose. Standard deviations were not shown in the plot and it was difficult to make a conclusion on the nebivolol effect on QTc based on this data presentation.

The reviewer performed an exploratory data analysis using the entire sponsor's data.

According to Figure 34, the mean changes in QTc observed on Day 7 at 10 hours post-dose were significantly higher than the same for the active control, atenolol. Figure 51 below shows the relationship between all QTcF changes vs. nebivolol plasma concentration obtained from the subjects on Day 7 at 10 hours post-dose. There is no trend in this plot.

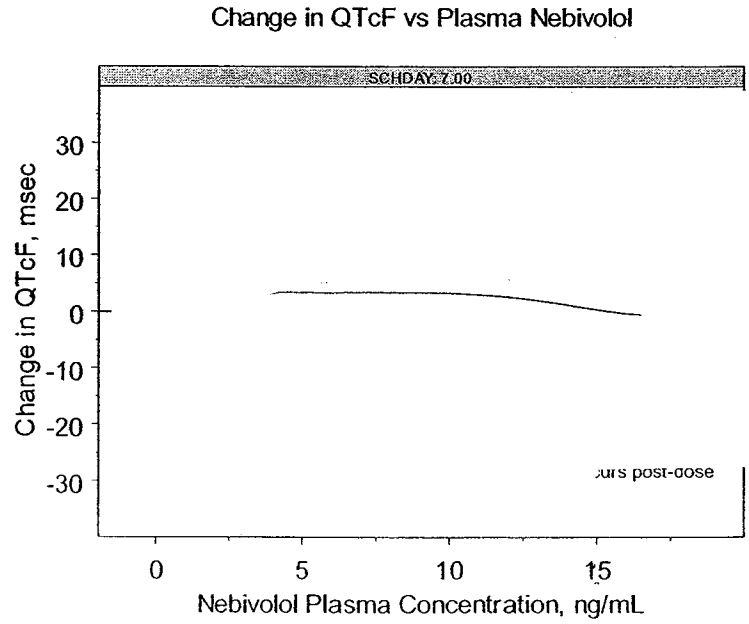

Figure 51: Change in QTcF vs. Plasma Nebivolol Concentrations