The sponsor's summary of anomalies is shown below.

Observation	Dosage group (mg / kg)			
	Control	2.5	10	40
A. Affecting the whole body	0	0	0	0
B. Affecting parts,regions or organs				
1. Cranium and contents	0	0	0	0
2. Spine and spinal cord	0	0	0	0
3. Face and sense organs	0	0	0	0
4. Neck	0	0	0	0
5. Thorax and contents				
- split center of the thoracic vertebra(e)	19	30	31	56 **
- rib(s)				
- one rudimentary 13th rib	0	0	0	1
- rudimentary 13th pair of ribs	0	0	0	2
- one rudimentary 14th rib	0	2	0	0
- sternum bone(s)				
- missing	1	0	1	0
- rudimentary	9	18	20	30 **
- asymmetrical	3	5	0	11 *
- dumbbell shaped	2	12 *	3	10 *
6. Abdomen and contents	0	0	0	0
7. Pelvis and contents	0	0	0	0
8. Urogenital system				
- ureter(s) twisted	44	28 **	38	26
- ureter(s) dilatation	8	17	16	12
- ureterohydronephrosis	0	0	0	1
9. Extremities	0	0	0	0

Significance computed by Mann-Whitney U test (two tailed) : * P < .05 ** P < .01 *** P < .001

Teratogenicity studies

HISTORICAL CONTROL DATA : LITTER DATA

Report	Live pups per litter n	Dead pups per litter n	Litter size n	Resorption sites n	Weight live pups Mean (g)
88-01	13.8 ± 1.1	0.05 ± 0.05	13.8 ± 1.1	0.57 ± 0.16	5.7
88-05	13.8 ± 0.8	0.04 ± 0.04	13.9 ± 0.8	0.42 ± 0.13	5.6
88-07	11.0 ± 1.2	0.1 ± 0.1	11.2 ± 1.2	1.06 ± 0.45	5.7
88-09	11.4 ± 1.3	0	11.4 ± 1.3	0.28 ± 0.16	5.6
88-10	11.1 ± 1.2	0	11.1 ± 1.2	0.43 ± 0.15	5.8
88-15	12.1 ± 1.0	0	12.1 ± 1.0	0.40 ± 0.15	5.5
88-17	14.1 ± 1.0	0	14.1 ± 1.0	0.35 ± 0.15	5.8
89-01	13.9 ± 0.7	0	13.9 ± 0.7	0.11 ± 0.07	5.9
89-03	13.4 ± 0.8	0	13.4 ± 0.8	0.14 ± 0.08	5.8
89-07	12.5 ± 1.0	0	12.5 ± 1.0	0.86 ± 0.53	5.4
89-08	12.5 ± 1.0	0.06 ± 0.06	12.6 ± 1.0	0.17 ± 0.17	5.8

Values are given as mean ± SE.

Study title: N51487 Oral embryotoxicity and teratogenicity study in New Zealand White rabbits (segment II)

Key study findings: The summary table for the dam and litter data is essentially unreadable. Summary data was taken from the text of the report. At the HD, 4 out of 13 litters were affected by skeletal anomalies, primarily abnormal thoracic vertebrae T10 and bifurcated or trifurcated ribs. As this is approximately 30% of the litters, I can't agree with the sponsor's conclusion that there were no drug related effects. The report also gives no indication of maternal toxicity at any dose.

Study no.: #1678 (86.08.12)

Conducting laboratory and location: Research Department, Janssen Laboratories, Aubervilliers, France
Date of study initiation: May 14, 1986
GLP compliance: statement included
QA reports: yes ( ) no ( )
Drug, lot #, and % purity: R67555, lot # V8510-201, in a vehicle of Avicel and Tween20

Doses of 0, 1.25, 5 and 20 mg/kg/day were given orally to NZW rabbits from GD6 through GD18. There were 15 females per group, fertilized by artificial insemination. The day of insemination was considered to be day 0 of pregnancy.

Observations
Dams: pregnancy
Clinical signs
Body weight
Mortality
Litter: litter size and weight at caesearean section
Number of resorptions, dead and live fetuses per litter
Abnormality

Results

One MD female died on day 27 with 9 fetuses. No cause of death was listed.

The summary table for the dam and litter data is almost entirely unreadable. The summary data is thus taken from the text of the report.

The tables for weight gain for the individual dosage groups are also poorly readable. The sponsor's summary of weight is taken from the text.

Body weight

The average weight gain of pregnant females (Table 1) for the various groups was:
- 357.9 g (controls)
- 192.3 g (1.25 mg/kg)
- 188.7 g (5 mg/kg)
- 296.2 g (20 mg/kg)

Initial and terminal body weight of all females is reported in the annexed tables 2 to 5. Although body weight decreased in all dosage groups no dose-related effect was seen since the same phenomenon occurred in the control group.

Food consumption was not recorded because of wastage.

Pregnancy

After artificial insemination the number of pregnancies in the dosed groups was 14/15 (1.25 mg/kg), 11/15 (5 mg/kg) and 13/15 (20 mg/kg) as compared with 14/15 in the control group. For the calculation, the pregnant females dead before sacrifice at the 28th day after insemination, were included.

Offspring

The number of live, dead and resorbed fetuses for the does sacrificed on the 28th day after insemination is given in Table 1. It is evident from this table that the average litter size in control does was 9.7, 6.2 ($p<0.01$) (1.25 mg/kg), 7.8 (5 mg/kg) and 6.8 ($p<0.01$) (20 mg/kg) in the various dosage groups. The number of live, dead and resorbed fetuses per female for all groups were respectively: 9.5 - 0.2 - 0.8 (controls), 6.1 ($p<0.01$) - 0.1 - 0.5 (1.25 mg/kg), 7.8 - 0.0 - 0.6 (5 mg/kg) and 6.8 ($p<0.01$) - 0.0 - 1.2 (20 mg/kg).

At resection, the birth weight of live pups (mean of the litter means) was: 35.9 g (controls), 42.0 g ($p<0.01$) (1.25 mg/kg), 39.5 g (5 mg/kg) and 39.4 g (20 mg/kg).

A decrease of the number of implantations was observed in all dosage groups resulting in a decrease of the number of living pups which was significant at the 1.25 and 20 mg/kg dose level but not at 5 mg/kg.

However, since the number of implantations in the control group is rather high for the used rabbit strain, the number of implantations in all dosage groups is considered to be within normal limits and not affected by the test article. No adverse effects on birthweigth were seen.

Gross observations
Fetal skeletal examination and fetal sectioning

The following abnormalities were seen :

Control group: 2/136
Female no. 4 : 1 fetus with abnormal thoracic vertebrae T8-9 and
loose rib
Female no.13 : 1 fetus with abnormal vertebrae and ribs T8-9-10

Low dosage group (1.25 mg/kg): 1/87
Female no.19 : 1 fetus with abnormal thoracic vertebrae T9-10

Medium dosage group (5 mg/kg): 1/78
Female no.31 : 1 fetus with abnormal thoracic vertebrae T9-10
(bifurcated ribs)

High dosage group (20 mg/kg): 6/89
Female no.51 : 1 fetus with abnormal thoracic vertebrae T10
1 fetus with trifurcated ribs
Female no.54 : 1 fetus with abnormal thoracic vertebrae T10
1 fetus with bifurcated ribs
Female no.55 : 1 fetus with abnormal thoracic vertebrae T10
Female no.59 : 1 fetus with bifurcated ribs

The normal control incidence of abnormalities in our rabbit colony is 1.6 %. A few abnormal thoracic vertebrae were seen in controls and dosed groups, the incidence of which was somewhat higher than normal at 20 mg/kg. However, since the same abnormalities were seen in both control and dosed groups we concluded that no drug-related abnormalities were seen.

At the high dose, 4 litters are affected by skeletal anomalies. As a percentage of 13 pregnancies, this is almost 30% of the litters. As this effect was also seen in the rat, I can't agree with the sponsor's conclusion that there were no drug related effects. It should also be noted that the report gives no indication of maternal toxicity at any dose.

Study Title: N71096 Embryotoxicity and teratogenicity study in New Zealand White rabbits (Segment II)

Key study findings: 6/15 HD dams died during the experimental period. Dosing at MD and HD resulted in a dose-related increased incidence of respiratory difficulties (8/15 MD and 12/15 HD). Signs were sneezing, coughing and sniffing. Some animals had no reported necropsy findings while others were found to have respiratory infections. This raises questions as to whether the effect is drug-related, dosing related or husbandry related. I do not agree that this necessarily means that the MD is maternally toxic. The presentation of fetal findings is not rich in detail or clarity of expression.

Study no.: N71096
Conducting laboratory and location: Research Department, Janssen Laboratories, Aubervilliers, France
Date of study initiation: January 18, 1989
GLP compliance: statement included
QA reports: yes ( ) no ( )
Drug, lot #, and % purity: R67555, batch PFA091 in a vehicle of HPβCD.

Methods

Doses of 0, 2.5, 5 and 10 mg/kg/day were given to NZW, 15 females to a group. The drug was given from GD6-GD18. The sponsor now states maternal toxicity as shown below.

The doses administered in this study were 0, 2.5, 5 and 10 mg/kg body weight/day. They were chosen based upon the available information on the toxicity of R 67555. In a previously conducted segment II study in rabbits (exp. 1678), R 67555 suspension was given at doses of 1.25, 5 and 20 mg/kg. No maternal toxicity, embryotoxicity or teratogenicity were observed. A pilot dosing trial with a solution of R 67555 in hydroxypropyl-β-cyclodextrin indicated pronounced clinical side-effects consisting of respiratory difficulties, dyspnea and sniffing in the female rabbits dosed at 20 mg/kg. Therefore it was decided to select 10 mg/kg as the highest dose in the present study, while 2.5 and 5 mg/kg were chosen as low and medium dose.

Observations

Clinical signs, mortality, weight of the uterus, number of corpora lutea, numbers of live and dead fetuses and embryos undergoing resorption. Live fetuses were weighed. Live and dead fetuses were examined for external anomalies. All fetuses were examined radiographically and dissected for organ examination. Bones were also stained with alizarin.

Results

The sponsor reported unscheduled morality and clinical signs that are shown in the entirety of the detail provided.

A. ADULT RABBIT DATA

CLINICAL OBSERVATIONS AND MORTALITY :

Control group :

Female no 3 (pregnant)	:	found dead on day 17 of pregnancy was apparently well the day before. Autopsy revealed pneumonia.
Female no 7 (pregnant)	:	delivered prematurely on day 23.
Female no 15 (pregnant)	:	found dead on day 15 of pregnancy previously showed anorexia. No specific macroscopic changes were noted at autopsy.

Low dosed group :

Female no 23 (pregnant)	:	had abnormal respiratory sounds on days 14 to 16 of pregnancy. No specific injuries were noted at autopsy.
Female no 28 (pregnant)	:	delivered prematurely on day 23.
Female no 30 (non pregnant)	:	had anorexia and bad general condition on day 19 of pregnancy. No specific injuries were noted at autopsy.

Medium dosed group :

Female no 37 (pregnant)	:	had abnormal repiratory sounds on day 10 of pregnancy. No specific injuries were noted at autopsy
Female no 38 (pregnant)	:	had abnormal respiratory sounds on days 10 to 14 of pregnancy. No specific injuries were noted at autopsy.
Female no 39 (pregnant)	:	had abnormal respiratory sounds on days 7 to 14 of pregnancy and delivered prematurely on day 20.
Female no 40 (pregnant)	:	found dead on day 19 of pregnancy had abnormal respiratory sounds, on days 7 to 18 additionally purulent nasal discharge was noticed on day 13. Autopsy revealed pneumonia.
Female no 41 (pregnant)		had abnormal respiratory sounds on day 15. No specific injuries were noted at autopsy

Female no 43 (pregnant) : had abnormal respiratory sounds on days 6 to 15 of pregnancy. No specific injuries were noted at autopsy.

Female no 45 (pregnant) : had abnormal respiratory sounds on days 7 to 15 of pregnancy. No specific injuries were noted at autopsy.

High dosed group :

Female no 46 (pregnant) : found dead on day 17 of pregnancy had respiratory distress before gavage the same day. Petechia on the stomach were noted at autopsy.

Female no 47 (pregnant) : had abnormal respiratory sounds on day 14 of pregnancy. No specific injuries were noted at autopsy.

Female no 48 (pregnant) : had abnormal respiratory sounds on days 10 to 14 of pregnancy . Autopsy revealed pneumonia.

Female no 49 (pregnant) : had abnormal respiratory sounds on days 11 to 15 of pregnancy. No specific injuries were noted at autopsy.

Female no 50 (not pregnant) : had abnormal respiratory sounds on days 10 to 19 of pregnancy. No specific injuries were noted at autopsy.

Females no 51 (pregnant) : found dead on day 17 of pregnancy was apparently well the day before. No specific injuries were noted at autopsy.

Female no 52 (pregnant) : had abnormal respiratory sounds on days 8 to 16 of pregnancy. Delivered prematurely on day 21.

Female no 53 (pregnant) : had abnormal respiratory sounds on days 7 to 17 of pregnancy. Autopsy revealed pneumonia.

Female no 55 (pregnant) : found dead on day 19 of pregnancy had abnormal respiratory sounds the days before (day 7 to day 18). Autopsy revealed pneumonia.

Appears This Way
On Original

Treatment : from day 6 through day 18 after mating

Embryotoxicity and teratogenicity in rabbit

Dosage groups :		R 67555		
	Control	2.5 mg	5 mg	10 mg
Adult rabbit data				
No. of dosed females	15	15	15	15
No. of dead females (1)	2/15	0/15	1/15	6/15
No. of pregnant females (1)	15/15	9/15 *	13/15	13/15
Body weight of pregn. fem. on d19 (2)	3953	3928	3903	3777
Body weight of pregn. fem. on d22 (2)	4014	4058	3982	3944
Weight change of pregn. females (2)	-27.8	-17.9	-163.6	-60.9
Food consumption (d 1 - d 5) (2)	904	890	985	1023
Food consumption (d 6 - d18) (2)	1885	2189	2122	1663
Food consumption (d19 - d27) (2)	1490	1379	1291	1498
Litter data				
Number of live pups / female (2)	6.8	8.5 *	8.0	5.7
Number of dead pups / female (2)	0.0	0.0	0.0	0.0
Mean litter size (2)	6.8	8.5 *	8.0	5.7
Number of resorptions / female (2)	1.0	0.8	0.7	1.6
Number of implantations / female (2)	7.8	9.3	8.7	7.3
Weight of live pups (caes. del.) (2)	32.0	30.7	31.2	33.9

Statistics : (1) Chi Square Test * p (0.05
(2) Mann-Whitney U Test ** p (0.01
*** p (0.001

Appears This Way
On Original

Observations	Dosage groups mg/kg			
	Control	2.5	5	10
No of fetuses (litters) examined	81 (12)	68 (8)	88 (11)	40 (7)
A. Affecting the whole body	0	0	0	0
B. Affecting regions, parts or organs				
- Thorax and contents				
. 13th ribs				
- extra pair	10	7	17	6
- rudimentary pair	3	2	14*	2
- unilateral rib	8	10	4	3
. Sternum bones				
- 2nd dumbbell-shaped	1	3	1	0
- 2nd rudimentary	0	0	2	1
- 4th dumbbell-shaped	0	0	1	0
- 5th missing	12	16	10	10
- 5th rudimentary	12	7	6	5
- 5th dumbbell-shaped	4	3	6	4
- 5th cleaved	0	1	0	0
- 6th missing	6	7	17	8
- 6th rudimentary	2	6	3	2
- 6th dumbbell-shaped	0	0	1	0
- 6th cleaved	0	2	0	0

Significance computed by Mann-Whitney U test (two tailed)

*p < 0.05
**p < 0.01
*** p < 0.001

N92570 (exp number 2383) Peri- and postnatal reproduction study with a second generation evaluation in Wistar rats (Segment III)

Key findings: Waiting until Day 21 to observe the stated developmental landmarks is a very insensitive method of evaluation. This study also did not identify a NOEL for decreased pup birth weight and decreased 21 day survival rate, consistent with the previous Seg III study. Carryover of effects into the second generation was not seen.

Study location : Janssen Research, Beerse, Belgium
GLP: statement included
QA: statement included
Study dates: Initiated June 16, 1991

Female Wistar rats were orally gavaged with Nebivolol in a βCD vehicle at doses of 0, 2.5, 10 and 40 mg/kg/day from GD18 through the 3 week lactation period.

In explaining the dose selection, the sponsor makes reference to another Seg III study, # 1888. In study #1888 maternal toxicity occurred at 10 mg/kg and was demonstrated in dystocia, cannabalism, lower number of live born pups and decreased survival. The same maternal effects were seen at 40 and 160 mg/kg/day with no surviving pups by PN day 4 in both groups.

JUSTIFICATION FOR SELECTION OF DOSES
In a previously performed segment III study with nebivolol (Exp.No.1888), doses were 10, 40 and 160 mg/kg. At 10 mg/kg, maternal toxicity, evidenced by dystocia and cannibalism, was associated with a lower number of live pups at birth and a decreased survival.
At 40 mg/kg, decreased body weight gain, dystocia and cannibalism resulted in no surviving pups after 4 days. Dosing at 160 mg/kg revealed a bad general condition with increased mortality, decreased body weight gain, dystocia and cannibalism. As a result, birth weight and survival rate decreased with no survivors after 4 days.
Based on these data, it was decided to reduce the doses in the present study and to select 2.5, 10 and 40 mg/kg body weight/day.

Postnatal observation of F1 animals: Litters were culled 3 weeks after birth. Where possible, 4 males and 4 females were kept for evaluation. The observations were as summarized by the sponsor:

Second Generation

Where possible, 1 male and three females of each litter were selected as parents of the next generation. Sibling mating was avoided. Males and females were paired on a 1:3 basis with cohabitation allowed for up to 3 weeks. On GD22 all females were euthanized. Radiographic examination was carried out for all fetuses of all groups. Half the fetuses were randomized for dissection and the other half for examination of the skeletal system. Standard observations were made.

Four male and four female pups in each litter were examined for the following physical developments during the period described below:

Pinna unfolding	day 21 after birth
Tooth eruption	day 21 after birth
Ear opening	day 21 after birth
Eye opening	day 21 after birth
Testis descent	day 42 after birth
Vaginal opening	day 42 after birth

Two male and two female pups in each litter were examined for the following behavioural developments during the period described below:

Righting on surface	day 21 after birth
Wire grasping	day 21 after birth
Walking	day 21 after birth
Righting in air	day 21 after birth
Climbing down a rope	day 21 after birth
Auditory startle	day 21 after birth
Pain response	day 21 after birth
Corneal reflex	day 21 after birth

At the age of ± 6 weeks, the horizontal activity was measured by Animex in five animals of each dosage group.

Results:

F0- Drug-treated

Unscheduled mortality was seen at the HD of 40 mg/kg. Five of the F0 HD females died. One of the dams died 2 days after parturition (dystocia). Two appear to have died during lactation period with respiratory difficulties. Two animals appear to have died of traumatic causes.

In the last 3 days of gestation the HD females gained on average 9% less than the control group (167g versus 185g) when the gain is calculated as a percentage of baseline. The HD group maintained a lower rate of gain throughout the lactation period also.

Pregnancy rate and gestation index were essentially unaffected. Duration of gestation in days was significantly increased in the HD group: 23.3days versus 22.8 days in the controls ($p<0.05$). There was a significant decrease in live pups and increase in dead pups in the HD group.

Dosage group mg/kg body weight/day	Mean number of pups per female live	dead
0	11.4	0.29
2.5	11.3	0.27
10	11.5	0.17
40	7.7***	2.35***

*** $p < 0.001$

Pups of all the treated mothers had lower body weights at all dosages and at all points of determination. The text of the report states that malnutrition was seen at all dose levels.

Survival was decreased in all treatment groups, significantly so in the MD and HD groups.

Treatment : from day 18 of pregnancy throughout lactation

		Control	2.5 mg	10 mg	40 mg
Adult rat data					
Number of dosed females		24	24	24	24
Number of dead (moribund) females	(2)	0/24	0/24	0/24	5/24
Number of pregnant females	(2)	24/24	22/24	23/24	24/24
Number of females with live-newborns	(2)	24/24	22/22	23/23	23/24
Body weight day 1 of pregnancy	(1)	201.5	195.9 *	201.4	200.6
Body weight day 18 of pregnancy	(1)	331.0	327.1	332.0	335.2
Body weight day 22 of pregnancy	(1)	386.7	382.3	385.6	367.6 *
Weight change (day 22 - day 18)	(1)	55.7	55.2	53.6	32.5 ***
Duration of gestation (days)	(1)	22.8	22.5 *	22.7	23.3 **
Body weight day of delivery	(1)	299.3	296.2	306.0	283.1 *
Body weight day 4 of lactation	(1)	311.0	308.6	311.7	280.8 **
Body weight day 14 of lactation	(1)	332.6	328.0	331.5	302.0 **
Body weight day 21 of lactation	(1)	321.6	323.5	324.7	317.1
Food consumption: pregnancy d 1 - d17	(1)	500.8	509.5	531.5	517.7
Food consumption: pregnancy d18 - d21	(1)	135.8	137.7	133.5	105.3 ***
Food consumption: lactation d 0 - d 3	(1)	159.3	150.2	136.7 **	85.7 ***
Food consumption: lactation d 4 - d13	(1)	602.8	560.7	527.8 *	254.9 ***
Food consumption: lactation d14 - d20	(1)	500.0	484.2	436.0 **	184.5 ***
Litter data					
Number of live pups / female	(1)	11.4	11.3	11.5	7.7 ***
Number of dead pups / female	(1)	0.29	0.27	0.17	2.35 ***
Mean litter size	(1)	11.7	11.6	11.7	10.0
Number of implantations	(1)	12.5	12.3	12.6	11.4
Body weight of pups at birth	(1)	6.7	6.3 *	6.0 **	6.1 **
Body weight of pups day 4	(1)	10.2	9.5	8.5 **	-
Body weight of males day 14	(1)	28.0	25.6	23.9 *	-
Body weight of females day 14	(1)	26.3	24.7	23.5	-
Body weight of males day 21	(1)	44.9	42.4	38.9	-
Body weight of females day 21	(1)	41.8	41.2	38.3	-
Survival rate after 4 days	(2)	89.7	90.4	77.7 ***	0.0 ***
Survival rate after 14 days	(2)	81.7	78.3	65.3 ***	0.0 ***
Survival rate after 21 days	(2)	81.7	76.7	63.4 ***	0.0 ***

(1) Significance computed by Mann-Whitney U Test (two tailed) : * p < .05 ** p < .01 *** p < .001
(2) Significance computed by Chi-Square Test (two tailed) : * p < .05 ** p < .01 *** p < .001

There were no reported developmental effects in ear opening, pinna reflex, auditory startle, eye opening, corneal reflex, tooth eruption, pain response, righting on surface, wire grasping, walking, righting in air, climbing down, testis descent and vaginal opening. Horizontal activity was presented as mean animex scores. The scoring was not defined.
Second generation: The number of live pups was slightly decreased and the number of dead pups was increased as summarized in the sponsor's table.

EXPERIMENT: 2383
Teratogenicity study
R 67555 - OR - RAT - Segment III - 2nd generation

! Summarized results : Pregnancy and litter

		Control	2.5 mg	10 mg
Adult rat data				
Number of dosed females		10	10	10
Number of dead females	(1)	0/10	0/10	0/10
Number of pregnant females	(1)	9/10	10/10	10/10
Body weight day 1	(2)	279.4	287.8	304.8
Body weight day 22	(2)	429.0	451.3	455.2
Weight gravid uterus	(2)	88.4	93.8	84.7
Weight change of pregn. females	(2)	61.1	69.7	65.7
Food consumption (d 1 - d 21)	(2)	619.9	641.6	672.2
Litter data				
Number of live foeti / female	(2)	12.0	12.2	11.4
Number of dead foeti / female	(2)	0.00	0.00	0.10
Mean litter size	(2)	12.0	12.2	11.5
Number of resorptions / female	(2)	0.56	1.20	0.80
Number of implantations / female	(2)	12.6	13.4	12.3
Number of corpora lutea / female	(2)	14.6	14.7	14.9
Weight of live foeti (caes.del.)	(2)	5.4	5.4	5.3
Sex ratio (%)	(2)	57.6	50.4	56.1
Number of malformed pups	(2)	0	0	2

(1) Significance computed by Chi Square Test (two tailed) : * p < .05 ** p < .01 *** p < .001
(2) Significance computed by Mann-Whitney U Test (two tailed) : * p < .05 ** p < .01 *** p < .001

Prenatal and postnatal development

Study title: Oral peri and postnatal study in Wistar rats (segment III)

Key study findings: This study did not identify a NOEL for maternal toxicity (decreased weight gain in the LD and MD groups; weight loss and death in the HD group), dystocia, cannibalism, poor pup survival and decreased weight gain in the surviving pups.

Study no.: N65774
Conducting laboratory and location: Janssen, Beerse, Belgium
Date of study initiation: January 11, 1988
GLP compliance: statement included
QA reports: yes (x) no ( )
Drug, lot #, and % purity: R67555 batch PFA031

Methods
The test article was administered daily admixed with the food from day 16 of pregnancy through a 3-week lactation period. The intended dose levels were 10, 40 and 160 mg/kg body weight per day. Four groups of 24 female rats of a Wistar substrain were used.
Parameters studied in the dams included clinical observations, mortality, body weight, food consumption, test article intake, pregnancy rate and duration of gestation. Litters were assessed for litter size, weight at birth and at 4,14 and 21 days of age. Litters were also assessed for number of live and stillborn fetuses per litter, survival of pups at 4, 14 and 21 days, and abnormalities.

Results
Clinical signs in the dams were reported only for the HD. Signs were poor general condition and ptosis. At parturition, dystocia and cannibalism were observed in each of the drug-treated groups but not the control group.

Unscheduled mortality was seen in all drug-treated groups: 0/24 (control), 2/24(LD), 3/24(MD), 8/24(HD).

Body weight effects were seen in all drug-treated groups. The sponsor's summary is reproduced below. The LD group gained 6% less weight than the control group from GD16-GD22 while the MD group gained on average 17% less. The HD group lost weight during this time period and continued to lose weight until time of parturition.

Average body weight recorded for the different groups

Dosage group mg/kg	Average body weight (g)					
	Pregnancy		Day of parturition	Day 4	Lactation	
	D 16	D 22			D 14	D 21
0	303.8	388.1	297.6	302.4	331.2	329.6
10	299.7	378.7 (-6)	296.5	296.5	317.5	321.0
40	305.9	375.8(-17)	289.8	274.9***	296.1***	311.6*
160	301.4	294.3***	237.4***	221.6***	207.4***	211.1***

*p<0.05, **p<0.01, ***p<0.001

On the day of parturition, the LD group weighed on average the same as the control group. Day 4 of lactation, the LD group weighed ~2% less than the control group while the MD group weighed 9% less. The HD group weighed 27% less than the control group. Food consumption was similar between the groups prior to dosing. During dosing, food consumption in the HD was decreased to approximately half that of the control group. During gestation, there was little difference between consumption in the control, LD and MD groups. During lactation however, there was a dose-related decrease in food consumption. This is summarized in the table.

Average food consumption (g)

Dosage group mg/kg	GD16-GD21	lactation
0	233.0	1241.7
10	222.8	990.9***
40	227.8	592.4***
160	132.4***	450.2***

***p<0.001

A slight increase in duration of gestation in the treated females

Dosage mg/kg	Duration of gestation (days)
0	23.0
10	23.1
40	23.3
160	23.2

Average numbers of live and dead pups per female at delivery

Dose (mg/kg)	# of pups per female		Average litter size
	live	dead	
0	10.8	0.58	11.4

10	8.7*	1.00	9.7*
40	8.0**	2.32***	10.3
160	6.7***	1.84***	8.5***

* p<0.05, **p<0.01, ***p<0.001

After this data, the sponsor reported that the increased number of dead pups, especially at the MD and HD was considered to be a consequence of the dystocia observed in some dams. Since litter size was established prior to dosing, the decrease in litter size was explained by an increased incidence of cannibalism at time of parturition. Cannibalism was also used to explain a lower number of dead offspring in the HD than the MD.

Average birthweight was lower in the treated groups. Surviving pup weight in the LD group was reported to be within normal limits, but was also consistently less than the control weights. Survival of the pups was decreased in all drug-treated groups: 50%(LD), 0%(MD) and 0%(HD). This occurred within the first 4 days of life. The sponsor reports that this was associated with maternal toxicity.

Average pup weight

Dose mg/kg	birth	Day 4	Day 14		Day 21	
			m	F	m	f
0	6.6	9.7	30.9	29.5	47.9	45.7
10	6.1*	9.5	29.3	27.3	45.6	42.8
40	5.9***					
160	5.1***					
Average survival rate(%)						
		Day 4	Day 14		Day21	
0		82.3	71.2		70.8	
10		64.0***	52.5***		50.0***	
40		0.0***	0.0***		0.0***	
160		0.0***	0.0***		0.0***	

*p<0.05, **p<0.01, ***p<0.001

OTHER

A telecon was held with the sponsor 10/22/02. The minutes of that telecon can be found in a separate memo. The substance of the discussion was that it was apparent that nebivolol causes an endocrine disruption as manifested by the reproductive and developmental toxicity studies, effects of decreased testicular weight in dogs in the 12 month study, Leydig cell tumors in mice, decreased absolute and normalized gonad weight in female mice and various poorly described effects on the female reproductive tracts ("a more resting appearance"). Amendment 097 was submitted subsequent to the telecon and among other things contained the studies N109053 and N106654, conducted to explore the endocrine effects. The material as it was submitted then was un-interpretable. The sponsor was requested to submit the results as tables of numbers for the reviewer to analyze.

3.4.7 Local tolerance

3.4.8 Special toxicology studies

Study title: Primary dermal irritation study in albino rabbits

Key study findings: Half a gram of R67555 applied to the backs of male albino rabbits for 3 days in an undescribed vehicle did not produce any reported skin effects.

Study no.: N84431
Conducting laboratory and location: Janssen, Beerse, Belgium
Date of study initiation: September 24, 1991
GLP compliance: statement included
QA reports: yes (x) no ( )
Drug, lot #, and % purity: R67555 no batch number was provided and the formulation of the material was not described.
Formulation/vehicle:

Methods An area was shaved on the backs of 3 male ——— albino rabbits. For each rabbit, 0.5 g of test article was applied to the skin under an occlusive dressing for 4 hours. The rabbits were then observed for 3 days. Dermal effects were scored according to the Draize system. Body weight was also monitored.

Results: No skin effects were reported for any of the observation periods.
All rabbits gained weight during the 3 days of the study.

Study title: Primary eye irritation study in albino rabbits

Key study findings: When 0.1g R67555 in an unspecified vehicle was applied to the conjunctiva of rabbits, one hour after conjunctival application the cornea of all rabbits became opaque and the iris showed congestion. Slight erythema and moderate chemosis were present in all rabbits. By 24 hours, severe discharge made further evaluation of some parameters impossible. Because of extreme eye irritation the rabbits were euthanized on day 1 to avoid further suffering.

Study no.:N84222/1
Conducting laboratory and location: Janssen, Beerse, Belgium
Date of study initiation: September 25, 1991
GLP compliance: statement included
QA reports: yes (x) no ( )
Drug, lot #, and % purity: R67555, batch PFA141, vehicle not specified

Methods: R67555 (0.1 g) in an undescribed vehicle was administered once in the left eye conjunctival sac of 3 male ——— albino rabbits. All animals were observed and scored for ocular reactions of the treated eye at 1 hour and 1 day after instillation with the untreated eye serving as control. Effects were scored by the Draize method.

Results: The sponsor's table of results is reproduced below.

	Score after 1 hour	Score after 24 hours
Rabbit 1		
Corneal opacity	2 discernible translucent areas, details of iris slightly obscured	3 opalescent areas, no details of iris visible, size of pupil barely discernible
Corneal opacity area	4 from ¾- whole cornea involved	4
iris	1 folds above normal, congestion, swelling, circumcorneal injection, sluggish to normal reactivity to light	1
Conjunctiva Redness Chemosis discharge	 1 definitely injected greater than normal 2 obvious swelling with partial eversion of lids 0	 n.d 3 3 moistening of lids and hairs and considerable area around the eye

Rabbit 2		
Corneal opacity	2	2
Corneal opacity area	4	4
Iris	1	1
Conjunctiva Redness Chemosis discharge	 1 2 0	 n.d. 2 3
Rabbit 3		
Corneal opacity	1	2
Corneal opacity area	4	4
iris	1	1
Conjunctiva Redness Chemosis discharge	 1 2 0	 n.d. 2 3

n.d. = scoring impossible

As described by the sponsor, one hour after conjunctival application the cornea of all rabbits became opaque and the iris showed congestion. Slight erythema and moderate chemosis were present in all rabbits. By 24 hours, severe discharge made further evaluation of some parameters impossible. Because of extreme eye irritation the rabbits were euthanized on day 1 to avoid further suffering.

N109053 Effects of daily oral administration of nebivolol and its enantiomers, R085547 and R085549 for 1 month on testicular and adrenal steroid biosynthesis in male and female mice.

Nebivolol and its enantiomers were admixed in the food of SPF Swiss mice at 20 and 40 mg/kg/day and administered for a period of 1 month. At the end of the treatment period, testicular cells of individual male mice were dispersed in cell culture medium and incubated in the presence of vehicle, ACTH and HCG. Plasma testosterone and corticosterone were determined by RIA. Ex vivo production of testosterone was determined by adding HCG or ACTH to the cell suspension. Testosterone was measured from the supernatant.

Results: There were no effects on body weight and no clinical signs reported except for some food wastage.

Serum corticosterone was decreased with R67555 and R85548 and increased with R85547. Corticosterone in males was increased at the HD with R67555 and R85548 and unchanged with R85547. Serum testosterone was increased both with the racemate and the enantiomers.

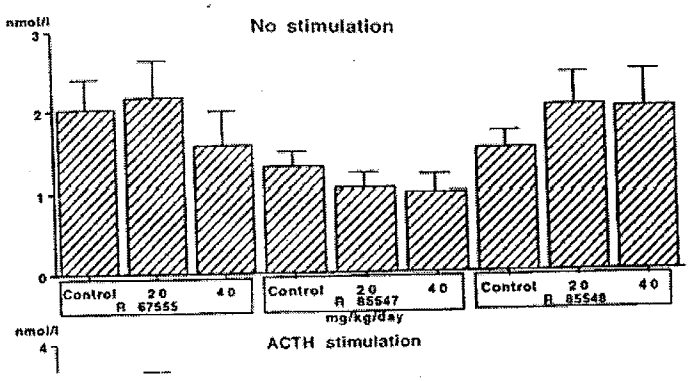
Summary of serum results

	Testosterone nmol/l	Corticosterone nmol/l

	R67555	R85547	R85548	R67555	R85547	R85548
Female						
Control				956±201	406±87	602±44
20 mg/kg				602**±144 p=0.004	671±450	522±121
40 mg/kg				709±290	505±132	388**±107 p=0.002
Summary of male results						
Control	7.9±7.6	3.6±3.4	6.4±4.0	555±126	313±83	207±110
20 mg/kg	7.9±6.8	6.0±11	26±48	578±122	347±102	291±66
40 mg/kg	12±19	8.2±13	21±23	734±286	276±143	339*±76 p=0.038

Serum testosterone levels increased in both racemate-treated animals and those treated with R85548 with a smaller increase in the R85547 group. In females, corticosterone values were decreased with the racemate and R85548. The opposite, an increase in corticosterone was seen in males treated with those two compounds.

There was no difference in the in vitro testosterone levels without stimulation and with ACTH. HCG stimulation caused an increase in ex vivo testosterone production but without statistically significant differences between the treatment groups. Results were expressed as mean ±SEM, indicating the variability of the system.



N109054 Effects of daily oral administration of nebivolol and its enantiomers, R085547 and 85548 for 1 month on ACTH- and LHRH-stimulated steroid biosynthesis in male mice.

Nebivolol and its enantiomers were given to male Swiss mice admixed in the food to give doses of 20 and 40 mg/kg per day for 1 month.

At the end of the 1-month dosing period, mice were weighed and injected intramuscularly with LHRH agonist buserelin (Receptal®, Hoechst, Germany) (40 ng/mouse) and tetrocosactidum (ACTH $_{1-24}$, Synacthen®, Ciba, Groot-Bijgaarden, Belgium) (25 µg/mouse) in a randomized way. Approximately 60 minutes later, the mice were sacrificed (while anaesthetized intraperitoneally with 40 mg pentobarbital/kg body weight) by exsanguination via the carotid artery. Blood was collected on heparin. Plasma was obtained after

Plasma testosterone was determined by RIA.

Results

Plasma testosterone increased with R67555 and R85547 ($p<0.001$) and decreased with R85548 ($p=0.012$). Plasma corticosterone increased non-significantly with the racemate and decreased with each enantiomer.

END 93/06 : NEBIVOLOL (Exp.nr : 2934-2963-2964)

	MB93/41	MB93/41	MB93/41	MB93/42	MB93/42	MB93/42
Treatment	**R67555**	**R85547**	**R85548**	**R67555**	**R85547**	**R85548**
	Testo	**Testo**	**Testo**	**Cortico**	**Cortico**	**Cortico**
	nmol/l	nmol/l	nmol/l	nmol/l	nmol/l	nmol/l
Control						
1	149	119	61	777	1298	1584
2	104	106	48	537	1692	889
3	102	120	84	728	1382	730
4	74	75	102	594	1243	1005
5	135	86	89	680	1216	1126
6	NS	87	87	NS	1403	615
7	81	107	89	667	1029	941
MEAN	*108*	*100*	*80*	*641*	*1323*	*984*
SD	*29*	*17*	*19*	*75*	*205*	*314*
SEM	*12*	*6.6*	*7.1*	*34*	*77*	*119*
20mg/kg/day						
21	178	241	96	631	1482	796
22	125	148	103	686	1362	733
23	121	113	99	720	1112	889
24	138	179	68	609	1003	715
25	109	118	106	722	931	776
26	159	162	86	582	1280	669
27	177	183	69	633	1411	740
MEAN	*144*	*163*	*90*	*655*	*1226*	*760*
SD	*28*	*44*	*16*	*55*	*212*	*70*
SEM	*10*	*17*	*5.9*	*21*	*80*	*27*
p versus control	*0.05*	*0.006**	*0.32*	*0.84*	*0.62*	*0.16*
40mg/kg/day						
41	136	157	46	734	1454	352
42	188	174	38	687	1395	532
43	249	147	22	588	1131	358
44	102	198	52	604	1034	438
45	136	210	80	773	1126	361
46	117	121	83	1554	1139	739
47	128	181	40	1115	1192	321
MEAN	*151*	*170*	*52*	*865*	*1210*	*443*
SD	*51*	*31*	*22*	*351*	*155*	*149*
SEM	*19*	*12*	*8.5*	*133*	*58*	*56*
p versus control	*0.14*	*<0.001***	*0.012***	*0.29*	*0.32*	*0.002***
SD : standard deviation; SEM : standard error of the mean						
Significance computed by Mann-Whitney U-Test						
* p≤0.05; ** p≤0.005						

N106654 Effects of daily oral administration of nebivolol and its enantiomers, R85547 and 85548 for 1 month on gluco- and mineralocorticoid function.

Nebivolol or the enantiomers were given via the diet to Wistar rats, 10/sex/group for 1 month at doses of 20 and 40 mg/kg.

Individual samples (max. 1 ml/rat) for determination of corticosterone and aldosterone levels, were drawn by puncture of the orbital venous plexus on days 1, 7 and 26 of the study. On day 26, 25 µg of ACTH 1-24 (Tetracosactidum, Synachten®, Ciba, Groot-Bijgaarden, Belgium) was administered subcutaneously, immediately after blood sampling. A second blood sample was obtained exactly 30 min. later. Blood was allowed to clot, centrifuged (10 min., 1850/g) and serum was stored at -20°C.

All rats were euthanized at the end of the 28-day dosing period.

Swollen adrenals were reported for the racemate (both doses in females), the group treated with R85548 (LD f, HD f, HD m) and R85547 (HD f).

This is one of the studies for which a table of numbers was provided. The reviewer's summary of results is shown below.

R67555: Corticosterone nmol/l

Males	Day 1	Day 14	Day 25 pre	Day 25 post
Control	456±154	798±102	672±129	2227±125
20 mg/kg	407±127	713±107	889±114	2362±129
40 mg/kg	336±127	634±138	361±70	1766±119* (p= 0.048)
Females				
Control	1145±256	1274±246	1471±300	3168±100
20 mg/kg	1085±203	1058±109	1694±191	2581±137* (p= 0.009)
40 mg/kg	903±240	788±90	850±129	1531**±149 (p=0.0019)

**, *p vs control by Mann-Whitney U test

R85547: Corticosterone nmol/l

Males	Day 1	Day 14	Day 25 pre	Day 25 post
Control	271±69	644±92	808±113	1930±74
20 mg/kg	239±81	480±69	564±121	1865±124
40 mg/kg	306±81	505±68	612±101	1772±73

Females				
Control	889±189	1174±248	1397±275	2637±49
20 mg/kg	693±178	1077±153	1329±244	2511±32 (p=0.059)
40 mg/kg	1437±265	1176±217	1313±236	2314*±66 (p=0.0028)

**, *p vs control by Mann-Whitney U test

R*5548: Corticosterone nmol/l

Males	Day 1	Day 14	Day 25 pre	Day 25 post
Control	222±103	538±81	491±72	1800±109
20 mg/kg	292±125	582±119	572±77	2028±161
40 mg/kg	Not given	522±43	594±87	1648±109
Females				
Control	643±206	1330±262	1615±294	2987±64
20 mg/kg	824±145	991±201	1211±131	2117**±113 (p=0008)
40 mg/kg	1024±201	740±118	947±116	1243**±150 (p=0.0002)

**, *p vs control by Mann-Whitney U test

R67555: Aldosterone nmol/l

Males	Day 1	Day 14	Day 25 pre	Day 25 post
Control	Values not provided			
20 mg/kg				
40 mg/kg	162*±28 (p=0.03)	849±313	345±75	2295**±249 (p=0.003)
Females				
Control	Values not provided			
20 mg/kg				
40 mg/kg	660±150	318**±55 (p=0.0012)	421±107	1230**±240 (p=0.0001)

**, *p vs control by Mann-Whitney U test

R85547: Aldosterone nmol/l

Males	Day 1	Day 14	Day 25 pre	Day 25 post
Control	313±53	661±106	400±77	3147±71
20 mg/kg	312±84	220±37	360±94	2905±147
40 mg/kg	260 ±56	369±89	346±96	2740±298
Females				
Control	406±78	857±218	646±229	3300±0

20 mg/kg	296±62	631±78	796±212	3300±0
40 mg/kg	563±122	526±133	424±93	3201±56

**, *p vs control by Mann-Whitney U test

R85548: Aldosterone pmol/l

Males	Day 1	Day 14	Day 25 pre	Day 25 post
Control	283±41	478±168	392±77	3089±135
20 mg/kg	255±43	347±131	301±57	2015**±151 (p=0.0006)
40 mg/kg	249±31	336±50	367±53	1770**±213(p=0.0007)
Females				
Control	582±123	1125±273	779±183	3205±63
20 mg/kg	430±70	448*±96	548±123	1682**±213 (p=0.0003)
40 mg/kg	641±101	481±132	419±93	1480**±239(p=0.0002)

**, *p vs control by Mann-Whitney U test

Within the variability of the assays, a statistically significant effect on aldosterone and corticosterone levels was seen with both enantiomers and the racemate in both males and females.

Animals treated with both racemate and enantiomers showed an increase in corticosterone following stimulation. However, the degree of increase was less than that of the control group, significantly so in most cases. Control and LD aldosterone values were not provided for the racemate. Treatment with R85547 did not affect the day 25 post-stimulation aldosterone results. A dose-dependent statistically significant decrease in day 25 post-stimulation aldosterone values was seen in both males and females treated with R85548. A decrease in plasma rennin was shown that is consistent with a beta adrenergic antagonist.

This single species, limited examination of the apparent endocrine effects indicates that there is indeed some endocrine effect but provides little in the way of characterization.

3.6 OVERALL CONCLUSIONS AND RECOMMENDATIONS

Conclusions: There are a number of consistent features in the toxicology studies. First, the level of detail provided is sub-optimal particularly with regard to histopathology results. In the majority of reports summary incidence tables are lacking. Instead, a scoring system is used which to some extent makes interpretation difficult. Detailed verbal descriptions of histopathology findings are almost entirely lacking. Similar comments may be made about some of the safety pharmacology studies where raw or interpretable results were not presented. Involved scoring systems were used making independent interpretation difficult.

Unresolved toxicology issues (if any):

b. N122168 Micronucleus test in mice: single oral dose. Single oral doses of nebivolol in male and female mice showed significant ($p \leq 0.05$-0.001) and dose-related reduction in bone marrow proliferation at the 24 hour sampling time. This bone marrow toxicity was not examined or at least there was no information in the toxicology reports characterizing or further exploring this finding. Decreases in HCT, Hb and RBC were seen in the hematology results of most toxicology studies. Enlarged spleens with increased RBC in the pulp were reported for most studies, even in situations where hematology changes were not apparent. The findings are more consistent with a hemolytic anemia rather than bone marrow depression. Was the original observation a random fluke?One would think that if the observation was real, that there might have been some clinical evidence to corroborate this finding by now. The points which should have been characterized include:
    a. Did the original effect repeat?
    b. A NOAEL for the bone marrow toxicity
    c. Is the effect reversible, progressive or self-limiting?

c. QTc prolongation. This appeared inconsistently. A consistent feature of the QTc evaluation was the lack of detail as to the determination of ECG collection relative to dosing. Also, Bazett's formula appeared to be the only method of correction used even though it was inappropriate given the heart rates of the dogs.

In the acute cardiovascular safety study there were no apparent effects on QTc. A 2 week repeat dose study in dogs also showed no QTc effects. A one month oral dosing study showed QTc increased in all groups including controls. One month of intravenous dosing showed a decrease in QTc values. A 3-month oral study showed inconsistent QTc increases from week 4 onward. A HERG assay indicated that nebivolol inhibits the IKr channel with an $IC_{50} = 3x10^{-7}M$ compared to astemizole, $IC_{50} = 2X10^{-8}M$ in the same assay

d. Endocrine disruption

This conclusion of endocrine disruption is due to several points of data:

a. a dose-related increase in Leydig cell tumors in mice. The LCT were assessed by the Executive CAC to be drug-related. LCT in mice are typically due to an estrogen receptor mechanism.
b. Several toxicology studies report weight effects in the reproductive organs of both sexes. Gross and histologic changes were also noted in report texts but detailed descriptions and incidences were not provided. For the female reproductive tract the sponsor notes " a more resting aspect in the female genital tract" as well as fewer corpora lutea and more atretic follicles. Changes in the male reproductive organs were noted in the 3 month study in mice and included Leydig cell hyperplasia(160 mg/kg), large nucleated tubular cells and testicular atrophy due to delayed maturation. Rat studies showed increased gonad weight (no detail as to the specifics). The 6-month rat study reported a decrease in gonad weight and testicular degenerative changes with low numbers of spermatozoa and "possible cellular debris in the epididymus." The one month dog study showed an increase in male prostate weight and no histopath information. The 3 month dog study showed increased goand weight at 2.5, 10 and 40 mg/kg with urolithiasis at the LD and prostatitis at the MD.

4) Reproductive toxicology was apparent.
Certain points from the reproductive and developmental toxicology studies are summarized below.

Reviewer's Summary of Findings

Study	Dosages (mg/kg)	Sponsor's statement of results
Seg I	0, 10, 40 and 160 premating, mating, to GD6	40 mg/kg: NOEL for fertility for both sexes according to sponsor, but not conclusive from the data presented.
Seg II	0, 2.5,10 and 40 GD6-GD16	40 mg/kg: Maternal toxicity characterized by decreased food consumption, decreased litter size, increased embryonal resorption, decreased pup weight
Seg III	0, 2.5, 10, 40 GD18-PN21	2.5 mg/kg: Decreased pup birth weight and decreased pup survival 10 mg/kg: Decreased food consumption during lactation as well as decreased pup weight and survival 40 mg/kg: Maternal toxicity characterized by mortality, ptosis, decreased body weight gain + food consumption, increased duration of gestation, decreased nursing behavior. No pups survived in this dosage group
Current Seg III	0, 1.25, 5 and 20	≥1.25 mg/kg decreased pup birth weight and decreased pup survival No surviving pups at the HD. Fertility was decreased in the F1 pups as shown by

		decreased number of implantations(≥1.25 mg/kg) and decreased number of corpora lutea per female (≥1.25 mg/kg). Maternal toxicity at 20 mg/kg as decreased weight

Study reports indicate cannabalism and dystocia but do not give specifics. The excerpt from N65774 is shown below as an example.

Dams

Clinical observations

No adverse effects at 10 and 40 mg/kg. Bad general condition and ptosis at 160 mg/kg. At the moment of parturition, dystocia and cannibalism were observed in some rats of the 10 mg/kg group and in several rats of the 40 and 160 mg/kg groups.

Comparison of species exposure: Surface area

Rat dose (mg/kg)	Human equivalent dose (mg/kg)	HED mg/m^2	Ratio of rat exposure/human(mg/m^2)
1.25	0.20	7.46	1.41
2.5	0.40	14.92	2.82
5	0.81	29.84	5.64
10	1.61	59.68	11.28
20	3.23	119.35	22.56
40	6.45	238.71	45.12
160	25.80	954.84	180.50

Human equivalent dose (HED) was calculated by dividing the rat dose by 6.2. The HED mg/kg was then multiplied by a Km of 37 to calculate the HED in mg/m^2.

Comparing exposure across species based upon plasma levels and AUC is more difficult due to the sponsor's contention that the data generated at the time of the studies underestimated the exposure. Single oral dose (gavage) studies in both rats and mice were conducted at 20 mg/kg. These were followed by 14 day dietary studies using 10 mg/kg. It is difficult to say from the data presented whether there is accumulation of drug, induction of metabolism and linear vs non-linear kinetics. We also have no data regarding differences, if any, in pregnant versus non-pregnant animals.

In normotensive rats, nebivolol did not lower blood pressure at an oral dose of 10 mg/kg. Therefore, reproductive effects seen at doses of ≤10 mg/kg would not be associated with profound alterations in blood pressure. A dose of 1-30 mg/kg i.p. will produce a lowering of the blood pressure in spontaneously hypertensive rats (SHR). Oral administration of 10 mg/kg nebivolol for 7 days will cause β1 adrenergic antagonism . However, the sponsor notes that in one strain of hypertensive rats the lowest orally effective dose was 10-20 mg/kg.

The sponsor also notes that doses of nebivolol up to 40 mg/kg i.p. "... failed to affect overt behavior (cornea-, pinna-, and tail withdrawal reflexes), or body functions (palpebral opening, pupil diameter, body temperature), apart from a slight decrease in body temperature and muscle tone; these latter effects were virtually absent after s.c. administration." This is suggestive that at oral doses ≤40 mg/kg, there should be little veterinary concern. However, maternal toxicity manifested by decreased food consumption was noted down to doses of 10 mg/kg p.o.

The developmental data was inconclusive due to the insensitive method used for evaluation. For example, eye opening was not examined until PN day 21 when the process may happen as early as PN 12. Vaginal opening was not examined until PN42 when it may be observed PN28. Righting on surface was not examined until PN21 but may be seen PN4.

As drug was administered later in gestation with each of the reproductive toxicology studies, the dose at which maternal weight gain was affected decreased. The F1 generation, pups whose dams were dosed with nebivolol, showed decreased birth weight and decreased survival in the first 21 days after birth. This effect repeated in two separate studies at doses of 2.5 mg/kg and 1.25 mg/kg. The exposure relative to the maximum recommended human therapeutic levels on a surface area basis was 2.8X and 1.4X respectively. When the untreated F1 pups became dams, corpora lutea, number of implantations and number of live fetuses was decreased in the offspring of F0 doses of 1.25 and 5 mg/kg.

Recommendations: At this point in development there may be enough human data to answer the various unresolved issues without the need for further animal studies.

Suggested labeling: See attached

Signatures (optional):

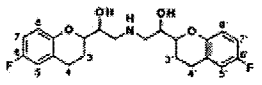
Reviewer Signature _____

Supervisor Signature_____ Concurrence Yes ___ No ___

3.7. APPENDIX/ATTACHMENTS

APPENDIX I: RECEPTOR BINDING PROFILE

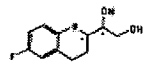
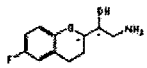
Table 1: Inhibition constants of nebivolol isomers and of hydroxylated derivatives for ^{125}ICYP binding to Huß$_1$AR and Huß$_2$AR expressed in *E. coli*.



nebivolol		SRRR/RSSS	purity		Huß$_1$AR a. pIC_{50} (M) [mean ± SD (n)] b $^*K_i$ (nM)		Huß$_2$AR a. pIC_{50} (M) [mean ± SD, n] b $^*K_i$ (nM)		ß$_1$/ß$_2$	Ratio of affinities d-isomer/l-isomer Huß$_1$AR	Huß$_2$AR
d-nebivolol	R 67 139	SRRR		a	9.40 ± 0.14	(4)	8.21 ± 0.09	(4)			
				b	0.21		2.50		12		
l-nebivolol	R 67 145	RSSS		a	6.99 ± 0.18	(5)	6.67 ± 0.17	(5)		257	35
				b	55		87		1.6		
7-OH	R 77 939	SRSS/RSRR RSSS/SRRR		a	9.57 ± 0.06	(3)	8.75 ± 0.05	(3)			
				b	0.14		0.72		5		
d-7-OH	R 77 939/1A	SRRR	>99%	a	9.75	(1)	9.1	(1)			
				b	0.098		0.32		3.4		
l-7-OH	R 77 939/1B	RSSS	>99%	a	8.5	(1)	8.0	(1)		18	13
				b	1.70		4.0		2.4		
5-OH	R 77 615	RSRR/SRSS RSSS/SRRR		a	9.45 ± 0.21	(2)	8.3 ± 0.1	(3)			
				b	0.19		2.0		11		
d-5-OH	R 77 615/1A	SRRR	>99%	a	9.3	(1)	8.4	(1)			
				b	0.27		1.6		6		
l-5-OH	R 77 615/1B	RSSS	>99%	a	7.9	(1)	7.5	(1)		25	8
				b	6.76		12.8		1.9		
5'-OH	R 77 612	SRSS/RSRR RSSS/SRRR		a	9.00 ± 0.14	(2)	7.91 ± 0.08	(2)			
				b	0.54		5.0		9.2		
d-5'-OH	R 77 612/1A	SRRR	>99%	a	9.3	(1)	8.5	(1)			
				b	0.27		1.28		4.7		
l-5'-OH	R 77 612/1B	RSSS	>96%	a	8.15	(1)	7.3	(1)		14	16 8
				b	3.8		20		5.3		

Appears This Way
On Original

Table 2: Inhibition constants of N-dealkylated nebivolol derivatives for ^{125}ICYP binding to Huß$_1$AR and Huß$_2$AR expressed in *E. coli*.

				Huß$_1$AR a. pIC_{50} (M) [mean ± SD, n] b $^*K_i$ (nM)		Huß$_2$AR a. pIC_{50} (M) [mean ± SD, n] b $^*K_i$ (nM)		
	R 80 269	RS/SR	a b	3.7 197000		< 4		
	R 80 330	RR/SS	a b	4.6 13500		3.8 64200		
	R 66 850	RS/SR	a b	6.18 ± 0.18 355	(2)	5.55 ± 0.21 1140	(2)	
	R 80 797	(+) SR	a b	5.96 ± 0.10 590	(2)	5.25 ± 0.14 2300	(2)	
	R 80 799	(-) RS	a b	6.03 ± 0.25 500	(2)	5.65 900	(1)	
	R 81 928	(-) RR	a b	5.00 ± 0.05 5400	(2)	4.0 40500	(1) (1)	
	R 82 844	(+) SS	a b	5.25 3020	(1)	4.75 7200		
	R 80 371	RS/SR SS/RR	a b	< 5		< 4		9

4-OH	R 82 251	RRSRR/SSRSS SSRRR/RRSSS			not available		not available				
d-4-OH	R 82 251/1A	SSRRR	>99%	a b	7.9 6.76	(1)	< 7	(1)			
l-4-OH	R 82 251/1B	RRSSS	>98%	a b	7.1 42.7	(1)	6.1 320	(1)	7.5	6.3	
4'-OH	R 82 244	SRSSS/RSRRR SRRRR/RSSSS		a b	8.6 1.35	(1)	7.35 18	(1)	13.4		
d-4'-OH	R 82 244/1A	SRRRR	>99%	a b	9.25 0.30	(1)	8.1 3.2	(1)	10.7		
l-4'-OH	R 82 244/1B	RSSSS	>95%	a b	7.8 8.5	(1)	6.6 64	(1)	7.5		28

*K_i values were calculated according to $K_i = IC_{50}/(1 + C/K_D)$ with C = 0.025 nM, the concentration of ^{125}I-CYP; K_D = 0.029 nM for Huß$_1$AR and K_D = 0.017 nM for Huß$_2$AR, the equilibrium dissociation constants of ^{125}I-CYP.

Appears This Way
On Original

NOVA Screen Results for Nebivolol compared to atenolol (1st column) and carvedilol(last column)

adrenergic subtype selectivity.

Assay	Atenolol	Nebivolol	Carvedilol
Adrenergic, Alpha 1, Non-selective	*	295	1.03
Adrenergic, Alpha 1A	*	2420	15.2
Adrenergic, Alpha 1B	*	389	4.79
Adrenergic, Alpha 2, Non-selective	*	948	557
Adrenergic, Alpha 2A (Human)	*	1540	10.9
Adrenergic, Alpha 2B	*	2140	42.8
Adrenergic, Alpha 2C	*	766	0.32
Adrenergic, Alpha 2C (Human Recombinant)	*	266	3.42
Adrenergic, Beta 1	757	0.91	0.31
Adrenergic, Beta 1 (Human)	442	0.12	0.16
Adrenergic, Beta 2	7330	70.2	0.76
Adrenergic, Beta 2 (Human Recombinant)	1610	4.5	0.05
Adrenergic, Beta 3 (Human)	*	620	57.9
Adrenergic, Beta, Non-selective	1940	19.8	1.37
Dopamine Transporter	*	4880	1360
Dopamine Transporter (Human Recombinant)	*	2850	403
Dopamine, D1	*	*	*
Dopamine, D1 (Human Recombinant)	*	4520	553
Dopamine, D2	*	1230	93.3

Assay	Ziprasidone	Risperidone	Haloperidol
Dopamine, D2s (Human Recombinant)	*	844	34.7
Dopamine, D3 (Rat Recombinant)	*	874	365
Dopamine, D4.2(Human Recombinant)	*	106	0.43
Dopamine, D4.4 (Human Recombinant)	*	56.2	0.56
Dopamine, D5 (Human Recombinant)	Not Available	Not Available	Not Available
Dopamine, Non-selective	*	3980	175
Dopamine, Non-selective (Clozapine)	*	*	3910
Serotonin Transporter	*	1310	1000
Serotonin Transporter (Human)	*	396	436
Serotonin, 5HT1	*	1180	77.7
Serotonin, 5HT1A	*	172	3.74
Serotonin, 5HT1A (Human Recombinant)	*	15.1	1.06
Serotonin, 5HT1B	*	179	10.0
Serotonin, 5HT1D	*	*	278
Serotonin, 5HT1D (Human)	*	*	855
Serotonin, 5HT2A	*	2170	251
Serotonin, 5HT2A (Human)	*	5380	579
Serotonin, 5HT2C	*	*	100
Serotonin, 5HT3	*	*	1040
Serotonin, 5HT4	*	*	114
Serotonin, 5HT5A (Human Recombinant)	*	*	184
Serotonin, 5HT6 (Human Recombinant)	*	881	422
Serotonin, 5HT7 (Human Recombinant)	*	1480	73.7
Serotonin, Non-selective	*	*	304
Estrogen	*	*	*
Glucocorticoid	*	*	*
Progesterone	*	*	*
Testosterone (cytosolic)	*	*	*
Potassium Channel, ATP-Sensitive	*	*	*
Corticotropin Releasing Factor, Non-selective	*	*	*
Oxytocin	*	*	*
Thyrotropin Releasing Hormone, TRH	*	*	*

Table I. Test articles and summarized assay data. Ki values in nM units. *Denotes Ki values > 10,000nM.

[illegible]

MOLECULAR PHARMACOLOGY, 34:843–851

The Receptor Binding Profile of the New Antihypertensive Agent Nebivolol and Its Stereoisomers Compared With Various β-Adrenergic Blockers

PETRUS J. PAUWELS, WALTER GOMMEREN, GUY VAN LOMMEN, PAUL A. J. JANSSEN, JOSÉE E. LEYSEN

Department of Biochemical Pharmacology (P.J.P., W.G., P.A.J.J., J.E.L.) and of Organic Synthesis (G.V.L., P.A.J.J.), Janssen Research Foundation, B-2340 Beerse, Belgium

Received June 2, 1988; Accepted August 23, 1988

SUMMARY

Nebivolol [the (*S,R,R,R*)- + (*R,S,S,S*)-racemic mixture], the 10 stereoisomers, and known β-adrenergic blockers were investigated *in vitro* for binding to β_1- and β_2-adrenergic receptor sites and various neurotransmitter, peptide, and ion channel binding sites and for inhibition of neurotransmitter uptake. Selective labeling of β_1- and β_2-adrenergic receptor sites in rabbit and rat lung, respectively, was obtained with [^{3}H]CGP-12177 and [^{3}H]dihydroalprenolol in the presence of an appropriate concentration of the selective β_2-adrenergic blocker ICI 118-551 or the selective β_1-adrenergic blocker CGP 20712-A. Nebivolol revealed high affinity and selectivity for β_1-adrenergic receptor sites in the rabbit lung membrane preparation (K_i value = 0.9 nM and β_2/β_1 ratio = 50). The drug dissociated slowly from these receptor sites. The activity resided in the (*S,R,R,R*)-enantiomer (R 67 138); the (*R,S,S,S*)-enantiomer (R 67 145) revealed 175 times lower β_1-adrenergic binding affinity. Within the series of stereoisomers, nebivolol and R 67 138 showed the best combination of high affinity and selectivity. Among the reference compounds, only CGP 20712-A shared these properties. Nebivolol bound to S_{1A} binding sites with a K_i value of 20 nM. The stereospecific requirements for interaction with these sites were different from those for the β_1-adrenergic receptor site. S_{1A} binding site affinity was also observed with the potent but nonselective β-adrenergic blockers carvedilol, pindolol, and propranolol. In the various other investigated radioligand binding and neurotransmitter uptake assays, nebivolol and its stereoisomers showed activity only at micromolar concentrations or were inactive. Clinical studies have shown an interesting hemodynamic profile of nebivolol, offsetting the negative effects on left ventricular performance generally observed with classical β-adrenergic blockers. Several hypotheses regarding the mechanism of action of nebivolol are summarized.

Nebivolol (R 65 824) (nebivolol-hydrochloride is R 67 555), (±)-[R^*[S^*-[S^*-(S^*)]]]-α,α′-[iminobis(methylene)]bis[6-fluoro-3,4-dihydro-2*H*-1-benzopyran-2-methanol], is a pseudosymmetrical molecule with four asymmetric carbon atoms (Fig. 1). Ten stereoisomers, comprising four enantiomeric pairs and two mesoforms, were synthesized and isolated.[1] Nebivolol, the racemic mixture of the (*S,R,R,R*)- and (*R,S,S,S*)-enantiomers, is being investigated as a new antihypertensive agent. In clinical studies with hypertensive patients, nebivolol was found to reduce heart rate and blood pressure but it also improved left ventricular function (1–3). In animal pharmacological studies, immediate reduction in blood pressure was observed with nebivolol, after its administration to conscious spontaneously hypertensive rats. No such effect was observed with known β-adrenergic blockers such as atenolol, propranolol, or pindolol. A further unusual feature observed at low doses of nebivolol was its apparent lack of negative cardiac inotropic effect in anesthetized dogs, in comparison with propranolol. Nebivolol reduced systemic vascular resistance and increased cardiac output and stroke volume. At equivalent doses, propranolol reduced cardiac output and stroke volume. Pharmacological investigations using isolated tissues have revealed a potent antagonism by nebivolol of isoprenaline-induced effects mediated by β_1-adrenergic receptors in the guinea pig atrium. However, the compound was 300-fold less active in antagonizing β_2-adrenergic receptor-mediated effects in the guinea pig trachea. A selective action of nebivolol at β_1-adrenergic receptors *in vivo* is apparent from the greater inhibition of isoprenaline-induced changes of left ventricular contractility mediated by cardiac β_1-adrenergic receptors, as compared with the reduction in diastolic blood pressure (vascular β_2-adrenergic receptors) in dogs (3).

Part of this work was supported by a grant from the Instituut voor Aanmoediging van het Wetenschappelijk Onderzoek in Nijverheid en Landbouw (Brussels, Belgium).

[1] Van Lommen et al., manuscript in preparation.

ABBREVIATIONS: CGP20712-A, (±)-[2-hydroxy-5-[2-[(2-hydroxy-3-[4-[(1-methyl-4-trifluoromethyl)1*H*-imidazole-2-yl]phenoxy]propyl)amino]ethoxy]-benzamide monomethane sulfonate; [^{3}H]CGP-12177, (±)-[^{3}H]4-(3-tertiarybutylamino-2-hydroxypropoxy)-benzimidazole-2-on hydrochloride; ICI-118551,erythro-1-(7-methylindan-4-yloxy)-3-isopropylaminobutan-2-ol.

Appears This Way On Original

Appears This Way
On Original

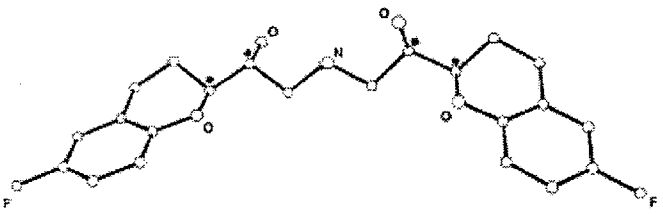


Fig. 1. Structure of nebivolol with indication (*) of the four asymmetric carbon atoms.

In this study, the receptor binding and neurotransmitter uptake inhibition properties of nebivolol were investigated. Specific radioligand binding models have been developed for selective labeling of β_1- and β_2-adrenergic receptor sites. This was achieved using (i) selective tissues, i.e., rabbit and rat lung for β_1- and β_2-adrenergic receptor sites, respectively, (ii) the selective β_1-adrenergic receptor blocker CGP 20712-A (4) and β_2-adrenergic receptor blocker ICI 118-551 (5, 6), and (iii) [^{3}H]CGP-12177 and [^{3}H]dihydroalprenolol as radioligands. The stereoselectivity of the β-adrenergic receptor interaction with the nebivolol stereoisomers was investigated. The dissociation rate of unlabeled drugs from the β_1- and β_2-adrenergic receptors was measured by modification of a previously described tissue-adsorbed-to-filter method (7, 8). The interaction of nebivolol stereoisomers with various neurotransmitter receptors, ion channels, and peptide binding sites was investigated. The potency of these compounds to inhibit monoamine uptake in rat brain synaptosomes was tested. The β-adrenergic selectivity and profile of nebivolol and its stereoisomers were compared with those of known β-adrenergic blockers. The biochemical profile of nebivolol is discussed in light of the reported pharmacological properties and findings in clinical studies.

Materials and Methods

Tissue preparation. Lungs from male New Zealand rabbits (~2 kg) and female rats (~150 g) were dissected and transferred in 0.9% NaCl. Tissue was homogenized in 10 volumes (volumes per wet weight tissue, v/w) of buffer (0.25 M sucrose, 0.15 M $NaClO_4 \cdot H_2O$, 5 mM EDTA, and 25 mM imidazol, pH 7.4) with a Polytron mixer (3 × 10 sec, 1500 rpm). The homogenate was centrifuged at 835 × g for 10 min to precipitate cell nuclei and debris. The pellet was rehomogenized and similarly centrifuged. The supernatants were combined, and filtered over cheesecloth, and further diluted up to 40 volumes per wet weight with 50 mM Tris·HCl, pH 7.7. This suspension was centrifuged at 23,600 × g for 20 min, to precipitate the cell membranes. The pellet was washed twice by suspension in Tris·HCl buffer and centrifuged. The final pellet was homogenized with a Dunli homogenizer in 10 volumes of 50 mM Tris·HCl, pH 8. During the entire preparation procedure the tissue suspension was kept at 0–4°. The membrane preparation was distributed into aliquots and stored at −70°. For binding assays, the membrane preparation was diluted to 100 volumes (v/w) with 50 mM Tris·HCl, pH 8.

Binding assays to β_1- and β_2-adrenergic receptor sites. Incubation mixtures were composed of 2 ml of tissue preparation, 0.1 ml of [^{3}H]CGP-12177 or [^{3}H]dihydroalprenolol, with or without drug for binding site occlusion, and 0.1 ml of solvent (10% ethanol), drug for inhibition, or drug for determination of nonspecific binding. Samples were mixed and incubated for 15 min at 37°. The reactions were stopped by adding 5 ml of ice-cold Tris·HCl buffer, pH 8.0, and rapid filtration over Whatman GF/B glass fiber filters under vacuum. The filters were rapidly rinsed twice with 5 ml of ice-cold Tris·HCl buffer, pH 8.0. Filters were placed in scintillation vials and radioactivity was extracted by vigorous shaking in 8 ml of Instagel II (Packard, Warrenville). The radioactivity was counted in a Packard Tri-carb 4530 liquid scintillation counter.

To measure binding to β_1-adrenergic binding sites in rabbit lung membranes, 10 nM ICI 118-551 was added with [^{3}H]CGP-12177 or [^{3}H]dihydroalprenolol for occlusion of β_2-adrenergic binding sites. Nonspecific binding was measured in the additional presence of 1 μM CGP 20712-A. To measure binding to β_2-adrenergic binding sites in rat lung membranes, 300 nM CGP 20712-A was added with [^{3}H]CGP-12177 or [^{3}H]dihydroalprenolol for occlusion of β_1-adrenergic binding sites. Nonspecific binding was defined in the additional presence of 1 μM ICI 118-551.

To measure potencies of drugs for inhibition of binding, 1 nM [^{3}H]CGP-12177 or [^{3}H]dihydroalprenolol was used. The drugs were added to the incubation mixtures in at least six concentrations, spanning 4 orders of magnitude. The specific [^{3}H]CGP-12177 or [^{3}H]dihydroalprenolol binding in the presence of drug was calculated as the percentage of total [^{3}H]CGP-12177 or [^{3}H]dihydroalprenolol binding and plotted versus the log of the drug concentration. IC_{50} values (concentration inhibiting 50% of specific [^{3}H]ligand binding) were derived graphically. K_i values were calculated according to the Cheng-Prusoff equation: $K_i = IC_{50}/(1 + C/K_d)$ with C being the concentration and K_d the equilibrium dissociation constant of the [^{3}H]ligand (9).

For saturation binding curves, [^{3}H]CGP-12177 or [^{3}H]dihydroalprenolol was used at concentrations between 0.05 and 1 nM. K_d and B_{max} values were derived from Scatchard plots. Linear regression lines were calculated by the method of least squares.

Measurement of β_1- and β_2-adrenergic receptor dissociation rates. The *in vitro* dissociation rates of the unlabeled drugs from the β_1- and β_2-adrenergic receptor sites were measured using a tissue-adsorbed-to-filter method as previously described (7, 8), with modifications. A tissue membrane preparation (see above), saturated with drug during preincubation with a concentration of 10 × IC_{50} value, was adsorbed to Whatman GF/B glass fiber filters positioned on the filtration apparatus. The drug-loaded tissues, adsorbed to the filters, were rinsed with warm buffer for various time periods. At the end of the rinsing period, the tissue, adsorbed to the filter, was incubated with a sample of [^{3}H]CGP-12177 to quantify free receptors. Calculation of the half-time of dissociation of the unlabeled drug was as previously described (7).

Radioligand receptor binding and neurotransmitter uptake assays. Radioligand binding assays were performed using rat or guinea pig brain membrane preparations (10). For neurotransmitter uptake, a crude synaptosomal fraction from rat brain regions was used (10). The assay conditions for serotonin S_2, serotonin S_{1A}, dopamine D_2, dopamine D_1, α_1-adrenergic, α_2-adrenergic, histamine H_1, cholinergic-muscarinic, κ opiate, benzodiazepine, dihydropyridine, biogenic amine, and metabolite release, substance P and neurotensin receptor binding, and serotonin, noradrenaline, dopamine and γ-aminobutyric acid uptake were as previously described.[*] Binding to the verapamil site of the

[*] Janssen, J. E., W. Gommeren, A. Eens, D. de Chaffoy de Courcelles, J. C. Stoof, and P. A. J. Janssen. Biochemical profile of raperidone, a new antipsychotic. *J. Pharmacol. Exp. Ther.* 247:661–670 (1988).

Appears This Way
On Original

Na^+ channel was measured with tetraphenylphosphonium ions as previously described (11).

Materials. (-)-[^{3}H]CGP-12177 (34 Ci/mmol) was from Amersham and *l*[propyl-1,2,3-^{3}H]dihydroalprenolol-HCl (48 Ci/mmol) was obtained from New England Nuclear (Dreieich, Germany). Nebivolol and stereoisomers were original substances from Janssen Pharmaceutica (Beerse, Belgium). Other drugs were generously supplied by the companies of origin.

Results

Development of receptor binding models for selective labeling of β_1- and β_2-adrenergic receptors. Inhibition of [^{3}H]CGP-12177 binding by the selective β_1- and β_2-adrenergic blockers CGP 20712-A and ICI 118-551, respectively, was measured in rabbit and rat lung membrane preparations; inhibition curves are shown in Fig. 2. In rabbit lung, CGP 20712-A showed a monophasic inhibition curve and inhibited 80% of total [^{3}H]CGP-12177 binding. The inhibition curve of ICI 188-551 was biphasic; it was noted that less than 15% of total bound [^{3}H]CGP-12177 was inhibited in the nanomolar range. In contrast, ICI 118-551 inhibited, at nanomolar concentrations, 80% of total [^{3}H]CGP-12177 binding in the rat lung membrane preparation. In this preparation, CGP 20712-A showed a biphasic inhibition curve; only 25% of the total [^{3}H]CGP-12177 binding was inhibited in the nanomolar range. These findings indicated that rabbit and rat lung membrane preparations were enriched in β_1- and β_2-adrenergic receptor sites, respectively. In subsequent experiments, the minor population of β_2- and β_1-adrenergic receptor sites in rabbit and rat lung was occluded by addition of 10 nM ICI 118-551 and 300 nM CGP 20712-A to rabbit and rat lung membrane preparations, respectively. Figs. 3 and 4 show the saturation binding curves of [^{3}H]CGP-12177 in rabbit and rat lung membrane preparations under such conditions. Scatchard analysis revealed a single population of binding sites in each of the tissues, representing β_1-adrenergic receptor sites in the rabbit lung and β_2-adrenergic receptors sites in the rat lung. Similar findings were obtained with [^{3}H] dihydroalprenolol. K_d and B_{max} values for [^{3}H]CGP-12177 and [^{3}H]dihydroalprenolol binding are summarized in Table 1. [^{3}H]

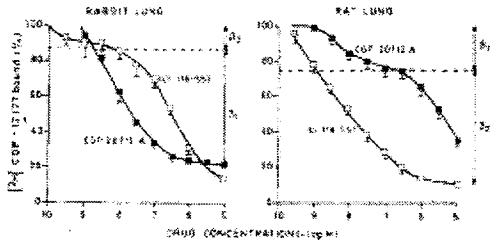


Fig. 2. Inhibition of total [^{3}H]CGP-12177 binding to rabbit and rat lung membrane preparations by CGP 20712-A and ICI 118-551. Binding was performed with 1 nM [^{3}H]CGP-12177 as described in Materials and Methods. In rabbit lung, total and nonspecific binding (in the presence of 1 μM CGP 20712-A) represent 16,690 ± 2,950 dpm and 3,713 ± 623 dpm, respectively. Rat lung, total and nonspecific binding (in the presence of 1 μM ICI 118-551) represent 17,511 ± 2,091 dpm and 2,547 ± 387 dpm, respectively. [^{3}H]CGP-12177 binding is expressed as percentage of total binding in the absence of unlabeled drugs. β_1, β_1-adrenergic receptor site; β_2, β_2-adrenergic receptor site. Curves were constructed using mean values ± standard error of three separate experiments performed in duplicate.

CGP-12177 bound with high affinity to β_1- and β_2-adrenergic receptor sites, the affinity for the β_1-adrenergic receptor being slightly higher. [^{3}H]Dihydroalprenolol bound with higher affinity to β_2- than β_1-adrenergic receptor sites. Its β_2-adrenergic receptor affinity was similar to that of [^{3}H]CGP-12177. The density of β_1-adrenergic receptor sites in the rabbit lung membrane preparation was equal to the density of β_2-adrenergic receptor sites in the rat lung membrane preparation. The receptor densities obtained with [^{3}H]dihydroalprenolol binding were in the same range.

Interaction of nebivolol, its stereoisomers, and various β-adrenergic blockers with β_1- and β_2-adrenergic receptor sites. Fig. 5 shows the inhibition curves of nebivolol, its *d*-enantiomer R 67 138 (*S,R,R,R*), and its *l*-enantiomer R 67 145 (*R,S,S,S*) on [^{3}H]CGP-12177 binding to β_1- and β_2-adrenergic receptor sites in rabbit and rat lung membrane preparations, respectively. Nebivolol and R 67 138 were as potent as CGP 20712-A in the inhibition of [^{3}H]CGP-12177 binding to rabbit lung membrane preparation. R 67 145 was 100 times less active than R 67 138. In contrast, [^{3}H]CGP-12177 binding to rat lung membrane preparation was only weakly inhibited by nebivolol and its two enantiomers. Nebivolol and R 67 138 were 100 times less potent than ICI 118-551 whereas R 67 145 was still 10 times less active. The eight remaining stereoisomers of nebivolol were similarly investigated; the binding affinities for β_1- and β_2-adrenergic receptor sites measured with [^{3}H]CGP-12177 and [^{3}H]dihydroalprenolol are summarized in Table 2. Nebivolol and R 67 138 showed the highest affinity for β_1-adrenergic receptors and they revealed a β_1/β_2 receptor selectivity of 40- to 50-fold. The most pronounced β_2-adrenergic selectivity (70–100-fold) was found with R 74 718 (*R,R,R,R*), but its β_1-adrenergic affinity was 12-fold less than that of R 67 138.

The β-adrenergic receptor binding affinity and selectivity of various known β-adrenergic blockers is shown in Table 3. Carvedilol, pindolol, and propranolol potently bound to β_1- and β_2-adrenergic receptor sites and lacked selectivity. Levantolol and labetolol were less potent and also nonselective. CGP 20712-A was potent and highly selective for β_1-adrenergic receptor sites whereas ICI 118-551 was a selective compound for β_2-adrenergic receptor sites. Atenolol showed low affinity for β_1- and β_2-adrenergic receptor sites and only moderate selectivity.

The dissociation rates of the compounds from the β_1- and β_2-adrenergic receptor sites were measured using the tissue-adsorbed-to-filter technique. The half-times of dissociation are presented in Table 4. Labetolol, pindolol, propranolol, and levantolol dissociated within a few minutes from both the β_1- and the β_2-adrenergic receptor sites. By contrast, nebivolol, R 67 138 (*S,R,R,R*), ICI 118-551, and carvedilol dissociated slowly from the β_1- and β_2-adrenergic receptor sites.

Interaction of nebivolol, its stereoisomers, and various β-adrenergic blockers with neurotransmitter receptors, ion channels, and peptide binding sites, and neurotransmitter uptake. The binding affinity *in vitro* of the nebivolol stereoisomers was measured in radioligand binding assays for neurotransmitter receptor sites, ion channels and peptide binding sites. The binding affinities of the stereoisomers expressed as $-\log IC_{50}$ values and K_i values are shown in Table 5. The potencies of the drugs ($-\log IC_{50}$) to inhibit the uptake of serotonin, norepinephrine, dopamine, and γ-aminobutyric acid

Appears This Way
On Original

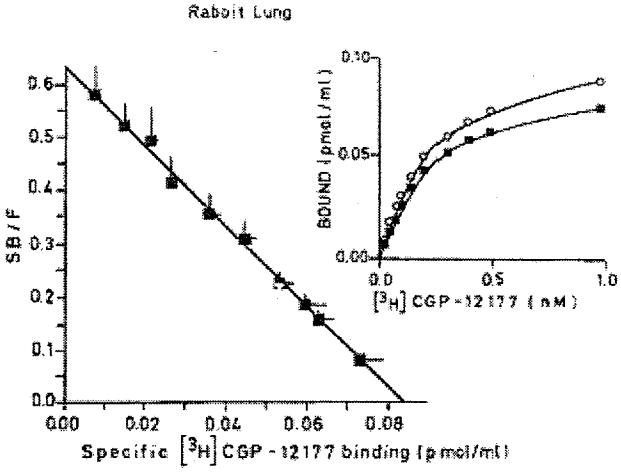


Fig. 3. Saturation binding curve (inset) and Scatchard plot of [^{3}H]CGP-12177 binding to β_1-adrenergic receptor sites in rabbit lung membrane preparation. Binding was carried out in the presence of 10 nM ICI 118-551 to block β_2-adrenergic receptor sites. Nonspecific binding was defined in the presence of 1 μM CGP 20712-A (○: total binding; ■, specific binding). Curves were constructed using mean values of binding data from four separate experiments. *SB*, specific [^{3}H]CGP-12177 binding; total bound [^{3}H]CGP-12177 in the presence of 10 nM ICI 118-551 minus nonspecifically bound. *F*, free [^{3}H]CGP-12177 concentration, added concentration of [^{3}H]CGP-12177 minus the total concentration bound. K_d value was given by the reciprocal value of the slope of the lines. B_{max} value was given by the intersection point with the abscissa (in pmol/ml). Lines were calculated using the method of least squares. Values are presented in Table 1.

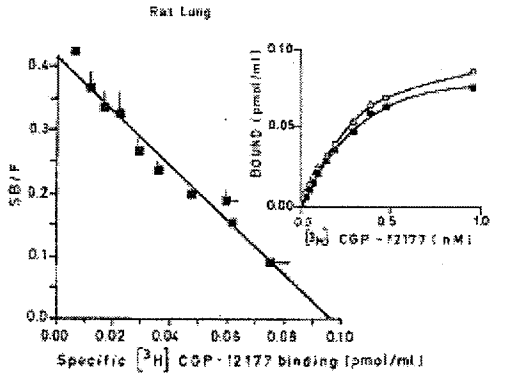


Fig. 4. Saturation binding curve (inset) and Scatchard plot of [^{3}H]CGP-12177 binding to β_2-adrenergic receptor sites in rat lung membrane preparation. Binding was carried out as described in the legend to Fig. 3 except that 300 nM CGP 20712-A was used instead of 10 nM ICI 118-551, to block β_1-adrenergic receptor sites. ○, Total binding; ■, specific binding. Derived K_d and B_{max} values are presented in Table 1.

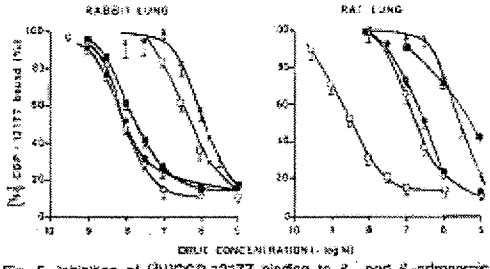


Fig. 5. Inhibition of [^{3}H]CGP-12177 binding to β_1- and β_2-adrenergic receptor sites in rabbit and rat lung membrane preparation, respectively, by nebivolol (○), R 67 138 (●), R 67 145 (×), CGP 20712-A (■), and ICI 118-551 (□). Rabbit lung binding was in the presence of 10 nM ICI 118-551; total binding, 13874 ± 1527 dpm; and nonspecific binding, 2475 ± 103 dpm. Rat lung binding was in the presence of 300 nM CGP 20712-A; total binding, 13384 ± 2199 dpm; and nonspecific binding, 1841 ± 228 dpm. [^{3}H]CGP-12177 binding is expressed as percentage of total binding in the presence of 10 nM ICI 118-551 and 300 nM CGP 20712-A for rabbit and rat lung, respectively. Curves were constructed using mean ± standard error values of three separate experiments in duplicate.

TABLE 1

K_d and B_{max} values of [^{3}H]CGP-12271 and [^{3}H]dihydroalprenolol binding to β_1- and β_2-adrenergic receptor sites in rabbit and rat lung membrane preparation.

K_d and B_{max} values are the means ± standard error of values obtained in four separate experiments.

	Rabbit lung β_1		Rat lung β_2	
	K_d	B_{max}	K_d	B_{max}
	nM	fmol/mg of tissue	nM	fmol/mg of tissue
[^{3}H]CGP-12177	0.14 ± 0.01	6.3 ± 0.7	0.24 ± 0.04	10.7 ± 0.7
[^{3}H]Dihydroalprenolol	0.89 ± 0.23	5.1 ± 1.3	0.21 ± 0.03	7.7 ± 0.4

in crude synaptosomal preparations are shown in Table 6. Several of the nebivolol stereoisomers bound to S_{1A} binding sites labeled with [^{3}H]8-hydroxy-2-(di-n-propylamino)tetralin; nebivolol, R 65 825, R 67 138, R 65 260, R 74 716, R 74 829, and R 67 142 showed K_i values between 20 and 40 nM. In the various other investigated radioligand binding and neurotransmitter uptake assays, nebivolol and its stereoisomers showed only activity at micromolar concentrations or were inactive.

In order to better visualize the profile of the nebivolol stereoisomers, pie charts have been constructed for nebivolol, R 67 138, and R 67 145 using the reciprocal of the K_i values for receptor binding and IC_{50} values for inhibition of monoamine uptake (Fig. 6). The pie chart shows the relative contribution of each activity in the sum of activities of the drug presented in Tables 2, 5, and 6. For nebivolol, β_1-adrenergic receptor binding accounts for 93%, β_2-adrenergic receptor binding for

TABLE 2

Binding affinity of nebivolol stereoisomers for β_1- and β_2-adrenergic receptor sites measured with two ligands

a, $-\log IC_{50}$ (M), mean value ± standard deviation. Numbers in parentheses, number of experiments. b, K_i values (nM). Binding was performed as described in Materials and Methods in the presence of 300 nM ICI 118-551 with rabbit lung membrane preparation and 10 nM CGP 20717-A with rat lung membrane preparation to measure β_1- and β_2-adrenergic receptor sites, respectively.

R-number, Configuration		[^{3}H]CGP-12177 binding (1 nM)			[^{3}H]Dihydroalprenolol binding (1 nM)		
		Rabbit lung β_1	Rat lung β_2	Ratio β_2/β_1	Rabbit lung β_1	Rat lung β_2	Ratio β_2/β_1
Nebivolol	a.	8.13 ± 0.05 (3)	6.6 (2)		8.72 ± 0.09 (4)	6.57 ± 0.05 (4)	
S,R,R,R + *R,S,S,S*	b.	3.88	46	55	0.91	44	48
R 65 825	a.	7.53 ± 0.05 (3)	6.15 ± 0.07 (3)		7.92 ± 0.09 (4)	6.13 ± 0.11 (3)	
S,R,S,S + *R,S,R,R*	b.	3.5	144	41	5.7	281	49
R 67 139	a.	8.17 ± 0.05 (3)	6.76 ± 0.05 (3)		8.95 ± 0.[illegible] (4)	6.96 ± 0.15 (3)	
S,R,R,R	b.	0.8	34	42	0.54	19	35
R 67 145	a.	5.93 ± 0.11 (3)	5.66 ± 0.05 (2)		6.53 ± 0.05 (3)	5.66 ± 0.11 (3)	
R,S,S,S	b.	140	423	3	138	367	2.6
R 65 260	a.	7.65 ± 0.07 (2)	6.70 ± 0.14 (2)		8.2 (2)	6.40 ± 0.14 (2)	
S,R,R,S	b.	2.7	39	15	3	68	23
R 74 715	a.	6.15 ± 0.07 (2)	5.85 ± 0.2 (2)		6.8 (2)	5.69 ± 0.07 (2)	
R,S,S,R	b.	84	433	5.15	75	375	5
R 74 829	a.	6.5 (2)	6.20 ± 0.14 (2)		7.05 ± 0.07 (2)	6.15 ± 0.2 (2)	
S,R,S,R	b.	38	122	3.2	43	125	2.9
R 74 714	a.	6.80 ± 0.14 (2)	5.95 ± 0.07 (2)		7.25 ± 0.07 (2)	6.0 (2)	
S,R,S,S	b.	30	217	7.2	27	166	6.1
R 67 142	a.	7.5 (2)	6.10 ± 0.14 (2)		7.90 ± 0.14 (2)	6.0 (2)	
R,S,R,R	b.	3.8	153	40	6	155	26
R 74 721	a.	5.95 ± 0.07 (2)	5.15 ± 0.07 (2)		6.40 ± 0.07 (2)	5.30 ± 0.14 (2)	
R,R,S,S	b.	133	1370	10	193	857	4.4
R 74 723	a.	5.1 (2)	5.30 ± 0.14 (2)		5.40 ± 0.14 (2)	5.19 ± 0.07 (2)	
S,S,S,S	b.	945	971	1	1925	1187	0.6
R 74 718	a.	7.05 ± 0.07 (2)	5.2 (2)		7.65 ± 0.07 (2)	5.35 ± 0.07 (2)	
R,R,R,R	b.	11	1222	111	11	744	68

TABLE 3

Binding affinity of various β-adrenergic blockers for β_1- and β_2-adrenergic receptor sites measured with two ligands

a, $-\log IC_{50}$ (M), mean value ± standard deviation. Numbers in parentheses, number of experiments. b, K_i value (nM). Binding was performed as described in the legend to Table 2.

		[^{3}H]CGP-12177 binding (1 nM)			[^{3}H]Dihydroalprenolol binding (1 nM)		
		Rabbit lung β_1	Rat lung β_2	Ratio β_2/β_1	Rabbit lung β_1	Rat lung β_2	Ratio β_2/β_1
CGP 20712-A	a.	7.66 ± 0.05 (3)	5.2		8.35 (2)	5.3 (2)	
	b.	1.6	1222	763	2.1	835	397
Atenolol	a.	5.65 ± 0.07 (2)	4.8 (2)		6.25 ± 0.07 (2)	4.75 ± 0.07 (2)	11
	b.	396	7493	19	266	2960	
Levantolol	a.	6.90 ± 0.07 (2)	6.60 ± 0.14 (2)		7.5 (2)	6.50 ± 0.14 (2)	
	b.	15	49	3.2	15	53	3.5
Labetolol	a.	5.7 (2)	7.0 (2)		7.40 ± 0.14 (2)	6.79 ± 0.07 (2)	
	b.	24	19	0.79	19	30	1.58
Carvedilol	a.	8.65 ± 0.07 (2)	9.0 (2)		9.02 ± 0.09 (4)	8.81 ± 0.02 (3)	
	b.	0.24	0.19	0.79	0.43	0.25	0.58
Pindolol	a.	8.13 ± 0.11 (3)	8.30 ± 0.1 (3)		8.66 ± 0.11 (3)	8.33 ± 0.15 (3)	
	b.	1.4	1.0	0.7	1.0	0.6	0.6
Propranolol	a.	7.83 ± 0.05 (3)	8.5 (2)		8.6 (2)	8.75 (2)	
	b.	2.8	0.62	0.22	1.2	0.29	0.24
ICI 118-551	a.	9.60 ± 0.14 (2)	8.60 ± 0.07 (2)		7.12 ± 0.09 (4)	8.43 ± 0.10 (3)	
	b.	48	0.49	0.01	35	0.62	0.02

1.7%, and S_{1A} binding site binding for 4.1%. The contribution of the other activities listed in Tables 5 and 6 is negligible. The relative contribution of each activity of R 67 138 (*S,R,R,R*) was similar. However, the chart of R 67 145, the (*R,S,S,S*)-enantiomer, which only weakly bound to β_1-adrenergic receptors, is completely different. For this compound, binding to S_{1A} sites accounts for 33%, β_1-adrenergic receptor binding for 22%, and β_2-adrenergic receptor binding, the veratridine site of the Na^+ channel, and the uptake of serotonine and dopamine between 7 and 10%.

The profile of various β-adrenergic blockers is shown in Tables 7 and 8. It reveals that carvedilol, pindolol, and propranolol also potently bind to S_{1A} binding sites with K_i values of 3, 15, and 84 nM, respectively. In addition, carvedilol and labetolol inhibited [^{3}H]WB 401 binding to α_1-adrenergic receptor sites with a K_i value of 3 and 42 nM, respectively.

Discussion

Specificity of the β_1- and β_2-adrenergic receptor binding model. In agreement with previous reports (see Ref. 12)

Appears This Way
On Original

Appears This Way
On Original

TABLE 4

Half-time of dissociation of nebivolol stereoisomers and β-adrenergic blockers from β_1- and β_2-adrenergic receptor sites

Values are mean ± standard deviation. Numbers in parentheses, number of experiments.

	$t_{1/2}$	
	Rabbit lung β_1	Rat lung β_2
	min	
Nebivolol	81 ± 19 (10)	32 ± 5 (11)
R 67 138	109 ± 35 (8)	37 ± 7 (4)
R 67 145	38 ± 8 (5)	7 ± 2 (3)
ICI 118-551	54 ± 3 (4)	47 ± 22 (6)
Carvedilol	47 ± 18 (9)	61 ± 28 (3)
CGP 20712-A	21 ± 6 (6)	
Levantolol	11 ± 6 (5)	7 ± 3 (2)
Atenolol	10 ± 3 (4)	
Propranolol	6 ± 1 (6)	13 ± 5 (3)
Pindolol	7 ± 1 (3)	7 ± 1 (2)
Labetolol	6 ± 2 (4)	5 ± 2 (2)

we found that rabbit lung was mainly enriched in β_1-adrenergic receptor sites and rat lung in β_2-adrenergic receptor sites. We obtained selective labeling of β_1- and β_2-adrenergic receptor sites in these tissues, respectively, with the nonselective radioligands [^{3}H]CGP-12177 and [^{3}H]dihydroalprenolol (Table 1), in the presence of an appropriate concentration of the selective β_2-adrenergic blocker ICI 118-551 or the selective β_1-adrenergic blocker CGP 20712-A. Using these conditions, Scatchard analysis of the binding data of the radioligands indicated the presence of only one binding site in the tissues, respectively (Figs. 3 and 4). The use of selective β_2- and β_1-adrenergic blockers for occlusion of the receptor sites, respectively, was recently also applied by Nunoff *et al.* (13) for selective labeling of β_1- and β_2-adrenergic receptor sites in rat cardiac microsomes.

Selectivity of nebivolol for β_1- and β_2-adrenergic receptor sites compared with its stereoisomers and other β-adrenergic blockers. Nebivolol was a potent blocker of β_1-adrenergic receptor sites in rabbit lung. It showed a subnanomolar K_i value of 0.9 nM, measured with [^{3}H]CGP-12177 as well as [^{3}H]dihydroalprenolol. Its *d*-enantiomer R 67 138 was equipotent whereas its *l*-enantiomer R 67 145 was 175-fold less potent. The least active stereoisomer, R 74 723 (*S,S,S,S*), showed a K_i value of 945 nM. CGP 20712-A, pindolol, propranolol, and carvedilol showed β_1-adrenergic affinity in the same range as nebivolol. In contrast, levantolol, labetolol, and atenolol were 17-, 27-, and 450-fold less active than nebivolol.

In addition, nebivolol showed a pronounced β_1-adrenergic selectivity inasmuch as it was 50 times less potent at β_2-adrenergic receptor sites in the rat lung membrane preparation. In the series of stereoisomers, the R 67 138 (*S,R,R,R*)-enantiomer showed the optimal combination of high potency and selectivity for β_1-adrenergic receptors. Nebivolol, which is a racemic mixture of the (*S,R,R,R*) and (*R,S,S,S*)-enantiomers, shared these properties. The inactive (*R,S,S,S*)-enantiomer apparently had little effect on the activity. The presence of this compound can only reduce the concentration of the active enantiomer by half. Using [^{3}H]dihydroalprenolol, the K_i value of nebivolol was indeed two times higher than that of R 67 138. This difference in K_i value was not observed with [^{3}H]CGP-12177, probably because the potency difference was within the experimental variation. Within the presently investigated series of β-adrenergic blockers, only two compounds showed combined high affinity and selectivity for β_1-adrenergic receptors; these were nebivolol and CGP 20712-A. The latter had a 2-fold lower affinity but was still 10 times more selective than nebivolol. Atenolol, generally referred to as a selective β_1-adrenergic blocker, was 300 to 400 times less potent and 2 to 4 times less selective than nebivolol. Striking differences were

TABLE 5

Receptor binding profile of nebivolol stereoisomers

a, −log IC_{50} (M), mean value ± standard deviation. Numbers in parentheses, number of experiments. b, K_i values (nM). Experimental details are described in Materials and Methods. Tested up to a concentration of 10^{-4} M, the nebivolol stereoisomers showed no interaction with dopamine D_1 receptors ([^{3}H]Sch 23390, rat striatum), cholinergic muscarinic receptors ([^{3}H]dexetimide, rat striatum), benzodiazepine receptors ([^{3}H]flunitrazepam, rat forebrain), μ-opiate receptors ([^{3}H]sufentanil, rat forebrain), substance P binding sites ([^{3}H]substance P, rat striatum), or neurotensin binding sites ([^{3}H]neurotensin, guinea pig forebrain). In addition, nebivolol showed a −log IC_{50} value of 6.3 (one experiment) for the biogenic amine and metabolite release site ([^{3}H]ketanserin, rat striatum).

		Inhibition of ^{3}H-ligand binding							
		Adrenergic α_1	Adrenergic α_2	Serotonin S_{1A}	Serotonin S_2	Histamine H_1	Dopamine D_2	Dihydropyridine binding site	Verapamil site of Na^+ channel
Nebivolol	a.	5.50 ± 0.07 (4)	<5.0	7.56 ± 0.05 (3)	5.66 ± 0.07 (2)	5.25 ± 0.07 (2)	5.0	5.75 ± 0.07 (2)	5.6
	b.	1160		20	700	2400	4000	589	2512
R 65 625	a.	5.55 ± 0.07 (2)	<6.0	7.30 ± 0.14 (2)	5.83 ± 0.1 (2)	5.07 ± 0.17 (2)	5.0	5.47 ± 0.24 (2)	5.42 ± 0.1 (2)
	b.	1033		35	780	3756	4000	1664	3802
R 67 138	a.	5.55 ± 0.07 (2)	5.00 ± 0.07 (2)	7.50 ± 0.08 (3)	5.70 ± 0.07 (2)	5.27 ± 0.03 (2)	5.34 ± 0.03 (2)	5.92 ± 0.03 (2)	5.20 ± 0.07 (2)
	b.	1033	4870	24	625	2900	1605	600	6310
R 67 145	a.	4.95 ± 0.07 (2)	<5.0	6.90 ± 0.08 (3)	5.20 ± 0.2 (2)	<5.0	5.37 ± 0.03 (3)	5.90 ± 0.14 (2)	5.42 ± 0.1 (2)
	b.	4115		95	2330		1650	629	3802
R 65 260	a.	5.65 ± 0.07 (2)	4.97 ± 0.03 (2)	7.3 (2)	5.37 ± 0.1 (2)	5.00 ± 0.1 (2)	5.25 ± 0.07 (2)	6.5 (2)	5.30 ± 0.14 (2)
	b.	820	8000	98	1328	4300	2222	158	5012
R 74 716	a.	4.52 ± 0.03 (2)	<5.0	7.27 ± 0.03 (2)	5.45 ± 0.14 (2)	<5.0	<5.0	6.30 ± 0.14 (2)	5.5 (2)
	b.	4300		40	1100			2505	3162
R 74 689	a.	5.32 ± 0.03 (2)	<5.0	7.35 ± 0.13 (3)	5.52 ± 0.17 (2)	<5.0	<5.0	5.55 ± 0.14 (2)	5.50 ± 0.14 (2)
	b.	1740		34	965			1409	5012
R 74 714	a.	5.6 (2)	<5.0	6.62 ± 0.1 (2)	5.27 ± 0.1 (2)	<5.0	5.22 ± 0.17 (2)	5.45 ± 0.2 (2)	5.37 ± 0.10 (2)
	b.	915		150	1573		2058	1774	4266
R 67 142	a.	5.35 ± 0.07 (2)	<5.0	7.42 ± 0.10 (2)	5.9 (2)	5.1 (2)	5.2 (2)	5.45 ± 0.07 (2)	5.37 ± 0.03 (2)
	b.	1620		26	990	3406	4000	1774	4266
R 74 721	a.	5.47 ± 0.03 (2)	4.92 ± 0.03 (2)	6.32 ± 0.1 (2)	5.71 ± 0.13 (2)	5.00 ± 0.07 (2)	5.3 (2)	5.70 ± 0.07 (2)	5.32 ± 0.17 (2)
	b.	1220	4400	380	522	4300	1980	997	4786
R 74 723	a.	<5.0	<5.0	6.05 ± 0.07 (2)	5.12 ± 0.1 (2)	<5.0	5.15 (2)	6.25 ± 0.07 (2)	5.37 ± 0.03 (2)
	b.			670	2323		2798	280	4266
R 74 718	a.	5.72 ± 0.03 (2)	<5.0	6.95 ± 0.07 (2)	6.55 ± 0.07 (2)	5.20 ± 0.07 (2)	<5.0	5.75 ± 0.13 (2)	<5.0
	b.	678		340	88	2704		589	

TABLE 6

Neurotransmitter uptake profile of nebivolol stereoisomers

a, $-\log IC_{50}$ (M), mean value ± standard deviation. Numbers in parentheses, number of experiments. b, IC_{50} (nM). Experimental details are described in Materials and Methods. Tested up to a concentration of 10^{-4} M, the nebivolol stereoisomers showed no interaction with [^{3}H]-γ-aminobutyric acid uptake in rat cortex.

		Inhibition of [^{3}H]-neurotransmitter uptake		
		Serotonin	Noradrenaline	Dopamine
Nebivolol	a.	6.47 ± 0.1 (2)	6.25 ± 0.07 (2)	6.40 ± 0.07 (2)
	b.	340	565	400
R 66 825	a.	6.57 ± 0.1 (2)	6.27 ± 0.03 (2)	6.65 (2)
	b.	269	531	223
R 67 138	a.	6.17 ± 0.03 (2)	6.00 ± 0.07 (2)	6.3 (2)
	b.	676	1000	501
R 67 145	a.	6.5 (2)	6.05 ± 0.07 (2)	6.37 ± 0.1 (2)
	b.	316	891	427
R 65 280	a.	6.02 ± 0.03 (2)	5.11 ± 0.17 (3)	6.35 ± 0.2 (2)
	b.	944	805	473
R 74 716	a.	6.15 ± 0.07 (2)	5.8 (2)	6.15 ± 0.2 (2)
	b.	712	1584	750
R 74 829	a.	6.15 ± 0.14 (2)	6.20 ± 0.14 (2)	6.52 ± 0.1 (2)
	b.	727	647	240
R 74 714	a.	6.70 ± 0.2 (2)	6.22 ± 0.17 (2)	6.4 (2)
	b.	211	620	398
R 67 142	a.	6.25 ± 0.2 (2)	5.97 ± 0.17 (2)	6.27 ± 0.1 (2)
	b.	562	1163	536
R 74 721	a.	6.47 ± 0.03 (2)	6.47 ± 0.03 (2)	6.37 ± 0.3 (2)
	b.	334	334	509
R 74 729	a.	6.25 ± 0.2 (2)	5.97 ± 0.1 (2)	6.12 ± 0.03 (2)
	b.	562	1074	758
R 74 718	a.	6.35 ± 0.07 (2)	6.35 ± 0.07 (2)	6.02 ± 0.03 (2)
	b.	446	1189	954

observed with the compounds in the half-times of dissociation from the β_{1}- and β_{2}-adrenergic receptor sites. Nebivolol can be considered a slowly dissociating drug from the β_{1}- as well as the β_{2}-adrenergic receptor sites. A slow drug receptor dissociation may increase the duration of action of the drug, although pharmacokinetic and metabolic processes may play a major role (7). The biggest advantage of slow receptor dissociation is the achievement of a stable receptor blockade, which is relatively insensitive to sudden variations in free concentrations of endogenous agonist or to fluctuations in the free concentration of the drug. This was also observed with R 67 138, ICI 118-551, and carvedilol whereas the other reference compounds dissociated within minutes from the β-adrenergic receptor sites.

Biochemical profile of nebivolol compared with its stereoisomers and other β-adrenergic blockers. Besides high binding affinity for β_{1}-adrenergic receptor sites, nebivolol bound to S_{1A} binding sites with a K_i value of 20 nM, i.e., about 20-fold higher than its K_i value for the β_{1}-adrenergic receptor site. Several of the nebivolol stereoisomers bound to the S_{1A} binding sites with a similar potency; hence, the stereospecific requirements for interaction with these sites appeared to be distinct from those for the β_{1}-adrenergic receptor site (Table 5). As a result, some of the stereoisomers bound more potently to the S_{1A} binding sites than to the β_{1}-adrenergic receptor sites. This was the case for the *l*-enantiomer of nebivolol, R 67 145 (*R,S,S,S*) (see Fig. 6), and for R 74 716 (*R,S,S,R*), R 74 829 (*S,R,S,R*) and R 74 723 (*S,S,S,S*). Carvedilol, pindolol and propranolol also bound to S_{1A} binding sites, although their β_{1}-adrenergic receptor affinity was at least 10-fold higher than their S_{1A} binding site affinity. At the same time, carvedilol showed α_{1}-adrenergic receptor affinity; it was 14-fold lower than its β_{1}-adrenergic affinity. Labetolol showed α_{1}-adrenergic affinity in the same range as its β_{1}-adrenergic affinity. Hence, it appeared that only CGP 20712-A, ICI 118-551, levantolol, and atenolol are characterized by β-adrenergic affinity free from other tested binding affinities.

Mode of action of nebivolol as antihypertensive agent. Clinical and *in vivo* pharmacological studies with nebivolol revealed an interesting hemodynamic profile, different from that of classical β-adrenergic blockers (see introduction). Observed reductions in heart rate can probably be attributed to β_{1}-adrenergic receptor blockade. However, improved left ventricular function, reduction in systemic vascular resistance, and related increased cardiac output seen with nebivolol are not properties of classical β-adrenergic blockers. Also, the immediate reduction in blood pressure, obtained after administration of nebivolol to conscious spontaneous hypertensive rats, has not been observed with known β-adrenergic blockers. Recent observations have revealed that the particular hemodynamic profile is specifically obtained with nebivolol, whereas the β_{1}-adrenergic active enantiomer R 67 138 (*S,R,R,R*) showed the activities of a typical β-adrenergic blocker. Hence, the properties of nebivolol apparently resulted from the combined activities of the two enantiomers. The presently investigated biochemical properties do not provide a direct clue for the explanation of the beneficial effects.

Drug properties reported to be related to decreased vascular resistance such as α_{1}-adrenergic or serotonin S_{2} antagonism,

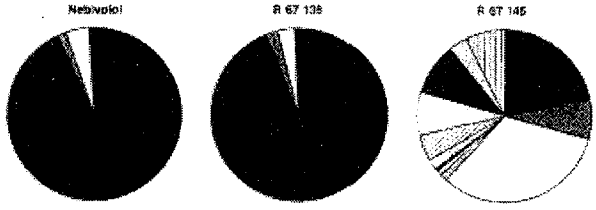


Fig. 6. Pie charts of the receptor binding and neurotransmitter uptake profile of nebivolol, R 67 138, and R 67 145. Pie charts were constructed using the reciprocals of K_i values in Table 2 and 5 and IC_{50} values in Table 6. The table shows the per cent contribution of each activity in the sum of the indicated activities of a nebivolol-stereoisomer. *DHP*, dihydropyridine binding site, *vera*, veratridine binding site of the Na^+ channel; *5-HT*, 5-hydroxytryptamine; *NA*, noradrenaline; *DA*, dopamine.

Appears This Way
On Original

TABLE 7

Receptor binding profile of various β-adrenergic blockers

a, −log IC_{50} (M), mean value ± standard deviation. Numbers in parentheses, number of experiments. b, K_i values (nM). Binding was performed as described in the legend to Table 5. Tested up to a concentration of 10^{-5} M, the β-adrenergic blockers showed no interaction with dopamine D_1 receptors ([^{3}H]Sch 23390, rat striatum), cholinergic muscarinic receptors ([^{3}H]dexetimide, rat striatum), benzodiazepine receptors ([^{3}H]flunitrazepam, rat forebrain), μ-opiate receptors ([^{3}H]sufentanil, rat forebrain), substance P binding sites ([^{3}H]substance P, rat striatum), or neurotensin binding sites ([^{3}H]neurotensin, guinea pig forebrain).

		Inhibition of ^{3}H-ligand binding							
		Adrenergic-α_1	Adrenergic-α_2	Serotonin S_{1A}	Serotonin S_2	Histamine H_1	Dopamine D_2	μ-Opiate	Veratridine site of Na^+ channel
CGP 20712-A	a.	<5.0	<5.0	5.10 ± 0.1 (3)	<5.0	<5.0	5.5 (2)	5.5 (2)	<5.0
	b.			595			992	854	
Atenolol	a.	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0	<5.0
	b.								
Levamolol	a.	5.66 ± 0.07 (2)	<5.0	6.3 (2)	5.42 ± 0.1 (2)	<5.0	<5.0	<5.0	<5.0
	b.	820		375	1,167				
Labetalol	a.	6.95 ± 0.08 (3)	<5.0	5.40 ± 0.07 (3)	<5.0	<5.0	<5.0	5.05 ± 0.07 (2)	<5.0
	b.	42		298				2,406	
Carvedilol	a.	8.03 ± 0.15 (3)	5.30 ± 0.05 (3)	8.36 ± 0.07 (2)	6.17 ± 0.03 (2)	5.15 ± 0.07 (2)	6.26 ± 0.05 (3)	5.00 ± 0.1 (3)	5.50 ± 0.2 (2)
	b.	3.4	2,166	3.4	237	3,034	213	2,700	
Pindolol	a.	5.3 (2)	<5.0	7.61 ± 0.1 (3)	<5.0	<5.0	<5.0	<5.0	<5.0
	b.	1,825		15					
Propranolol	a.	<5.0	<5.0	6.95 ± 0.07 (2)	5.1 (2)	<5.0	<5.0	<5.0	
	b.			84	2,466				
ICI 118-551	a.	<5.0	<5.0	4.8 (2)	5.25 ± 0.07 (2)	<5.0	<5.0	<5.0	<5.0
	b.			18,849	1,629				

TABLE 8

Neurotransmitter uptake profile of various β-adrenergic blockers

a, −log IC_{50} (M), mean value ± standard deviation. Numbers in parentheses, number of experiments. b, IC_{50} (nM). Uptake was performed as described in the legend to Table 6. Tested up to a concentration of 10^{-5} M, the β-adrenergic blockers showed no interaction with [^{3}H]γ-aminobutyric acid uptake in rat cortex.

		Inhibition of [^{3}H]-neurotransmitter uptake		
		Serotonin	Noradrenaline	Dopamine
CGP 20712-A	a.	5.60 ± 0.14 (2)	5.35 ± 0.07 (2)	<5.0
	b.	2,577	4,468	
Atenolol	a.	<5.0	<5.0	<5.0
	b.			
Levanlolol	a.	6.63 ± 0.1 (3)	6.55 ± 0.14 (2)	5.52 ± 0.03 (2)
	b.	235	288	2,374
Labetolol	a.	5.75 ± 0.07 (2)	6.0 (2)	5.52 ± 0.1 (2)
	b.	1,769	1,000	3,029
Carvedilol	a.	6.30 ± 0.15 (4)	5.62 ± 0.1 (2)	6.23 ± 0.2 (3)
	b.	526	2,406	627
Pindolol	a.	5.0 (2)	5.61 ± 0.07 (3)	5.1 (3)
	b.	10,000	2,417	7,943
Propranolol	a.	5.95	5.50 ± 0.07 (2)	5.1
	b.	1,122	3,160	7,943
ICI 118-551	a.	5.72 ± 0.03 (3)	5.48 ± 0.17 (4)	5.35 ± 0.07 (2)
	b.	1,850	3,250	4,460

Ca^{2+} entry blockade, or dopamine D_2 antagonism were not observed with nebivolol or the stereoisomers. In this study, nebivolol and both separated enantiomers showed S_{1A} binding site affinity; however, this affinity was also observed with carvedilol, pindolol, and propranolol. S_{1A} binding sites have been proposed to have a role in cardiovascular function. Hypotension and bradycardia have been observed with 8-hydroxy-2-di-N-propylamino-tetralin, considered as the prototype of S_{1A} agonists, in very particular experimental conditions (14, 15). However, the reported cardiovascular effects of this S_{1A} agonists are unlike the hemodynamic effects of nebivolol. Neither did carvedilol, pindolol, and propranolol share the hemodynamic effects of nebivolol. In the absence of a sufficient number of drugs that specifically act as agonists and antagonists on the S_{1A} binding sites in the absence of controlled clinical data, hypotheses on the role of the S_{1A} binding sites must be regarded with caution. Several hypotheses regarding the mechanism of action of nebivolol can be proposed. One possibility is an antagonistic action at presynaptic β-adrenergic receptors involved in the release of adrenaline and noradrenaline from adrenergic neurones (16). Otherwise, emerging molecular biological data on the amino acid sequences of cloned receptors have revealed dissimilarities in the sequence of the β-adrenergic receptor obtained from various tissues and species (17, 18). Therefore, it seems not unlikely that different, as yet unidentified, receptor subtypes exist, which may have particular functions. Such possibilities could be explored with nebivolol.

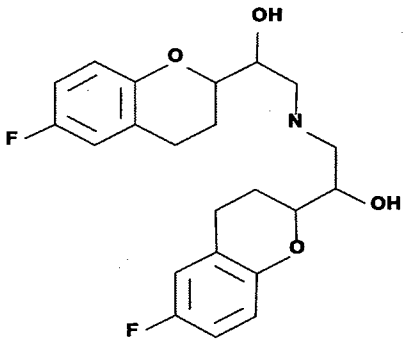
Acknowledgments

We sincerely thank Suzy De Geewer for secretarial work.

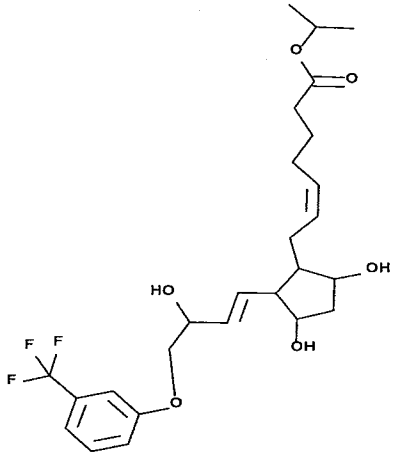
References

1. De Cree, J., H. Geukens, J. Leempoels, and H. Verhaegen. Haemodynamic effects in man during exercise of a single oral dose of nebivolol (R 67 555): new beta-1-adrenoceptor blocking agent: a comparative study with atenolol, pindolol, and propranolol. Drug Dev. Res. 8:109–117 (1986).
2. De Cree, J., H. Geukens, C. Cobo, and H. Verhaegen. Subacute hemodynamic effects of nebivolol in man at rest and during exercise. Angiology 38:440–448 (1987).
3. Van de Water, A., W. Janssens, J. Van Neuten, R. Xhonneux, J. De Cree, H. Verhaegen, R. S. Reneman, and P. A. J. Janssen. The pharmacological and hemodynamic profile of nebivolol, a chemically novel, potent and selective β_1-adrenergic antagonist. J. Cardiovasc. Pharmacol. 11:552–563 (1988).
4. Dooley, D. J., and H. Bittiger. Quantitative assessment of central β_1- and β_2-adrenoceptor regulation using CGP 20712-A. J. Pharmacol. Methods 18:131–136 (1987).
5. O'Donnell, S. R., and J. C. Wanstall. Evidence that ICI 118-551 is a potent, highly beta-selective adrenoceptor antagonist and can be used to characterize beta-adrenoceptor populations in tissues. Life Sci. 27:671 (1980).
6. Bilski, A., S. Dorries, D. Fitzgerald, R. Jessup, H. Tucker, and J. Wale. ICI 118-551, a potent $beta_2$-adrenoceptor antagonist. Br. J. Pharmacol. 69:P292 (1980).
7. Leysen, J. E., and W. Gommeren. The dissociation rate of unlabelled dopamine antagonists and agonists from the dopamine-D_2 receptor application of an original filter method. J. Recept. Res. 4:817–845 (1984).
8. Leysen, J. E., and W. Gommeren. Drug-receptor dissociation time, new tool for drug research: receptor binding affinity and drug-receptor dissociation profiles of serotonin-S_2, dopamine-D_2, histamine-H_1 antagonists, and opiates. Drug Dev. Res. 8:119–131 (1986).
9. Cheng, Y.-C., and W. H. Prusoff. Relationship between the inhibition constant (K_i) and the concentration of inhibitor which causes 50 per cent inhibition (I_{50}) of an enzymatic reaction. Biochem. Pharmacol. 22:3099–3108 (1973).
10. Leysen, J. E., P. Van Gompel, W. Gommeren, R. Woestenborghs, and P. A. J.

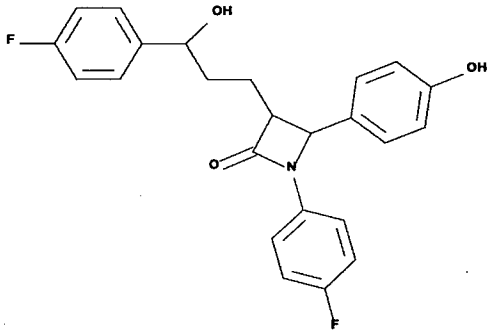
APPENDIX II ICSAS J. F. Contrera Consult for Elizabeth Hausner 11/23/04
Nibivolol [sic] Pharmaceuticals Similarity Search (Cosine Method)
Full Battery of MDL-QSAR 2D Descriptors. (Top 5)



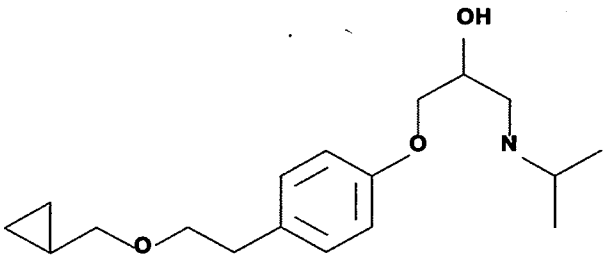
Nibivolol Test Compound = 100%



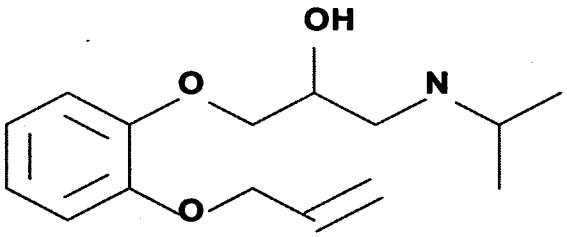
Travoprost: Similarity = 86%



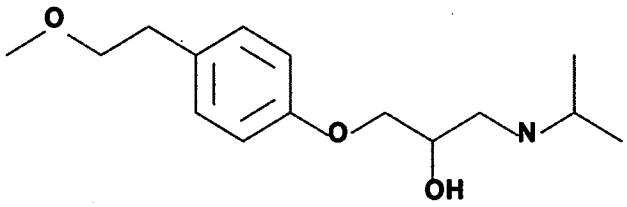
Ezetimbe: Similarity = 81%



Levobetaxolol: Similarity = 80%



Oxprenolol: Similarity = 79%



Metoprolol: Similarity = 79%

3 Page(s) Withheld

b Trade Secret / Confidential

_____ Draft Labeling

_____ Deliberative Process

Withheld Track Number: Pharm/Tox-_____

27 Page(s) Withheld

_____ Trade Secret / Confidential

6 Draft Labeling

_____ Deliberative Process

Withheld Track Number: Pharm/Tox-_____

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Elizabeth Hausner
1/10/05 01:07:37 PM
PHARMACOLOGIST
Elizabeth Hausner

Albert Defelice
1/24/05 04:22:56 PM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY REVIEW

3.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21742
Review number: 1
Sequence number/date/type of submission: 0/November 12, 2004/
Information to sponsor: Yes ( ) No ( )
Sponsor and/or agent: Bertek
Manufacturer for drug substance: Mylan Pharmaceuticals Inc, 781 Chestnut Ridge Road, Morgantown, WV 26505.

Reviewer name: **Elizabeth Hausner, D.V.M.**
Division name: **Cardio-Renal Drug Products**
HFD #: 110
Review completion date: December 1, 2004

Drug:

Generic Name: Nebivolol Tablets

Chemical Name Nebivolol hydrochloride is identified chemically as (±)-[2R*[R*[R*(S*)]]]-α,α′-[iminobis(methylene)]bis[6-fluoro-3,4-dihydro-2H-1-benzopyran-2-methanol] hydrochloride

Code Numbers R067555

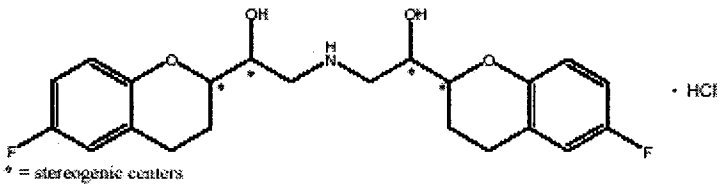
R067138 (d-Nebivolol)

R067145 (l-Nebivolol)

CAS Registry No. 152520-56-4

Trade Name: To Be Established

Figure 3.2-01 Chemical Structure of Nebivolol



Empirical Formula $C_{22}H_{25}F_2NO_4 \cdot HCl$

Molecular Weight 441.90 g/mol

Due to 4 chiral carbons, there are 10 different stereoisomers possible. The drug substance is the racemate of the enantiomeric pair SRRR-nebivolol (d-nebivolol) and RSSS-nebivolol (l-nebivolol).

Studies submitted

XBL study number 02852, XBL report number RPT00830 Isolation and Structural Determination of [^{14}C]-Nebivolol Glucuronides from Human Urine

XBL study # 03825, XBL Report # RPT01030 Identification of Nebivolol Metabolites Generated from Human Liver Microsomes

XBL study # 03829, XB: report # RPT01075 Isolation, Purification, and Identification of Nebivolol Metabolites from Human Feces

XBL study # 04683, XBL report # RPT01128 *In vitro* Metabolism of [^{14}C]-Nebivolol in Liver Microsomes, and Liver S9 Fraction from Mouse, Rat, Dog, and Human

XBL study # 04684, XBL report # RPT01132 *In vitro* Glucuronidation of [^{14}C]-Nebivolol Using Human Liver and Kidney Microsomes and UDP-Glucuronosyl Transferases (UGT)

XBLStudy # 04685, XBL report # RPT01174Search and Investigation of Nebivolol Metabolites in Plasma Samples from Human, Rat, Mouse, and Dog Using Liquid Chromatography-Tandem Mass Spectrometry (LC/MS/MS) techniques

Studies Reviewed

XBL study # 04683, XBL report # RPT01128 *In vitro* Metabolism of [^{14}C]-Nebivolol in Liver Microsomes, and Liver S9 Fraction from Mouse, Rat, Dog, and Human

XBLStudy # 04685, XBL report # RPT01174Search and Investigation of Nebivolol Metabolites in Plasma Samples from Human, Rat, Mouse, and Dog Using Liquid Chromatography-Tandem Mass Spectrometry (LC/MS/MS) techniques

XBL study # 04683, XBL report # RPT01128 In vitro Metabolism of [^{14}C]-Nebivolol in Liver Microsomes, and Liver S9 Fraction from Mouse, Rat, Dog, and Human

Study location: [redacted]
Study dates: initiated June 2004
GLP: no
QA: no
Test article:

Compound Name	Lot Number	Purity
d-nebivolol:	S46730/1	
l-nebivolol:	S46731/1	[redacted]
[^{14}C]-*d*-nebivolol:	X1505:19A	
[^{14}C]-*l*-nebivolol:	X1505:19B	

[^{14}C]-d and [^{14}C]-l-nebivolol were separately incubated with 1) liver microsomes from male and female mice, rats, dogs and humans and 2) liver S9 fractions from male mice, rats, dogs and humans. Incubations were carried out for 37°C, 2 hours at a concentration of 10µM. Incubation mixtures were extracted with methanol and centrifuged. The methanolic extracts were profiled by HPLC. "Prominent" metabolites were characterized by LC/MS. Levels of radioactivity were determined by liquid scintillation counting.

Results

Overall, the in vitro metabolic profiles of [^{14}C]-d and [^{14}C]-l-nebivolol were qualitatively similar.

As may be seen in the sponsor's summary below, there were quantitative differences in the degree of metabolism reported for the systems used for the different species.
The mouse tissues showed the least degree of metabolism with 34-75% unchanged parent drug present. Rats tissues showed 9-40% unchanged drug present. Dog tissues showed the greatest degree of metabolism with 0-20% unchanged drug remaining. Quantitatively, the human tissues were closest to the rat and mouse with 15-80% unchanged drug remaining. Mouse and rat microsomes showed quantitative differences in the amount of d- and l-nebivolol remaining. Qualitatively, these metabolites are represented across the species.

Percent Distribution of Radioactivity of the Prominent Metabolites in Methanol Extracts from 2-Hour Liver Microsomal and Liver S9 Fraction Incubations with 10 μM of [^{14}C]-*d*-, and [^{14}C]-*l*-Nebivolols

Test System	Substrate*	Metabolite Code						
		A-5	A-6	A-8	A-9	A-10	A-11	Neb
	d	7.20	2.25	26.83	3.09	4.80	4.59	33.95
	l	6.08	3.33	27.50	2.69	2.82	7.86	42.78
	d	8.66	2.38	28.84	3.18	5.47	6.78	41.46
	l	7.83	4.01	26.57	2.22	3.63	7.97	45.09
	d	0.51	0.24	21.17	+	ND	0.34	74.51
	l	1.60	5.34	19.94	1.84	ND	1.17	68.24
	d	18.14	5.31	38.40	2.71	7.47	4.91	9.40
	l	11.35	2.67	45.84	4.35	3.32	6.63	23.13
	d	4.67	0.43	61.41	+	0.38	1.90	30.11
	l	5.19	ND	45.82	3.45	ND	1.70	43.57
	d	6.17	1.96	62.02	+	0.94	1.29	24.53
	l	5.21	2.03	40.14	5.74	1.04	2.03	39.84
	d	1.20	44.86	19.49	1.60	1.14	5.41	13.39
	l	+	31.78	25.45	1.17	3.15	12.68	15.73
	d	4.91	20.03	16.37	1.42	4.71	11.70	6.90
	l	2.30	2.36	15.20	+	8.91	16.97	3.97
	d	+	35.71	14.13	1.07	2.31	6.60	0.60
	l	2.84	5.57	12.87	0.74	5.13	11.19	0.48
	d	5.66	7.80	22.42	3.82	7.97	14.59	21.03
	l	12.68	12.81	28.26	2.38	1.26	10.30	20.98
	d	6.53	6.92	19.15	2.58	9.72	18.01	17.23
	l	6.36	16.45	24.83	2.99	5.68	13.31	18.99
	d	3.19	10.60	27.20	4.26	2.00	6.09	35.87
	l	1.33	22.40	32.05	+	0.82	3.51	15.47

+: Present, but can't be integrated due to overlapping; ND: Not Detectable. *d: [^{14}C]-*d*-Nebivolol and *l*: [^{14}C]-*l*-Nebivolol.

Appears This Way
On Original

Metabolites Identified or Characterized following Incubation of [^{14}C]-*d*-Nebivolol with Liver Microsomes from Male Mice, Rats, Dogs, and Humans

Metabolite Code	Test System			
	[redacted]			
A-1	ND	ND	Figure 37 & Figure 41	Figure 37 & Figure 41
A-3	Figure 41	Figure 41	Figure 37 & Figure 41	Figure 37 & Figure 41
A-4	ND	ND	ND	ND
A-5	Figure 37 & Figure 43	Figure 37 & Figure 43	Figure 37 & Figure 43	Figure 37 & Figure 43
A-6	Figure 20	Figure 20	Figure 20 & Figure 24	Figure 20 & Figure 24
A-7	ND	ND	ND	Figure 45
A-8	Figure 20 & Figure 24	Figure 20 & Figure 24	Figure 20 & Figure 24	Figure 20 & Figure 24
A-9	Figure 30	Figure 30	Figure 30	Figure 30
A-10	Figure 50	Figure 50	Figure 50	Figure 50
A-11	ND	Figure 54	Figure 54	Figure 54
A-12	Figure 57	ND	Figure 57	ND

ND: Not Detectable

Metabolites Identified or Characterized following Incubation of [^{14}C]-*l*-Nebivolol with Liver Microsomes from Male Mice, Rats, Dogs, and Humans

Metabolite Code	Test System			
	[redacted]			
A-1	ND	ND	Figure 38, & Figure 42	Figure 38, & Figure 42
A-3	Figure 38, & Figure 42	Figure 38, & Figure 42	Figure 38, & Figure 42	Figure 38, & Figure 42
A-4	Figure 31	ND	ND	ND
A-5	Figure 38, & Figure 44	Figure 38, & Figure 44	Figure 38, & Figure 44	Figure 38, & Figure 44
A-6	Figure 21, & Figure 27	Figure 21	Figure 21, & Figure 27	Figure 21, & Figure 27
A-7	Figure 46	Figure 46	ND	Figure 46
A-8	Figure 21, & Figure 27	Figure 21, & Figure 27	Figure 21, & Figure 27	Figure 21, & Figure 27
A-9	Figure 31	Figure 31	Figure 31	Figure 31
A-10	Figure 51	Figure 51	Figure 51	Figure 51
A-11	Figure 55	Figure 55	Figure 55	Figure 55
A-12	Figure 58	ND	ND	ND

ND: Not Detectable

The sponsor's comparative summary of metabolites is shown below.

Appears This Way On Original

Metabolites Identified or Characterized following Incubation of [^{14}C]-*d*-Nebivolol with Liver S9 Fraction from Male Mice, Rats, Dogs, and Humans

Metabolite Code	Test System			
	[redacted]			
A-1	ND	ND	Figure 39	Figure 39
A-3	ND	Figure 39	Figure 39	Figure 39
A-4	ND	ND	ND	ND
A-5	Figure 39	Figure 39	Figure 39	Figure 39
A-6	Figure 22	Figure 22	Figure 22, & Figure 26	Figure 22, & Figure 26
A-7	ND	Figure 47	ND	ND
A-8	Figure 22, & Figure 26	Figure 22, & Figure 26	Figure 22, & Figure 26	Figure 22, & Figure 26
A-9	Figure 32	Figure 32	Figure 32	Figure 32
A-10	NA	NA	NA	NA
A-11	NA	NA	NA	NA
A-12	ND	ND	ND	ND

NA: Not Applicable.
ND: Not Detectable

Metabolites Identified or Characterized following Incubation of [^{14}C]-*l*-Nebivolol with Liver S9 Fraction from Male Mice, Rats, Dogs, and Humans

Metabolite Code	Test System			
	[redacted]			
A-1	ND	ND	Figure 40	Figure 40
A-3	ND	ND	Figure 40	Figure 40
A-4	Figure 33	ND	ND	ND
A-5	Figure 40	Figure 40	Figure 40	Figure 40
A-6	Figure 23, & Figure 29	Figure 23	Figure 23	Figure 23, & Figure 29
A-7	ND	Figure 48	ND	ND
A-8	Figure 23, & Figure 29	Figure 23, & Figure 29	Figure 23, & Figure 29	Figure 23, & Figure 29
A-9	Figure 33	Figure 33	Figure 33	Figure 33
A-10	NA	NA	NA	NA
A-11	NA	NA	NA	NA
A-12	ND	ND	ND	ND

ND: Not Detectable
NA: Not Applicable

Appears This Way On Original

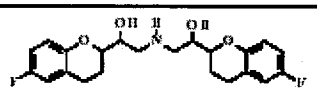
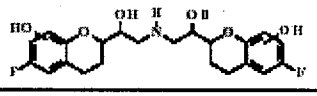
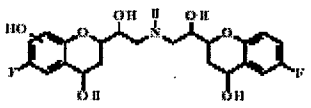
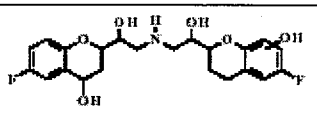
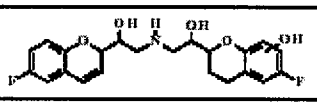
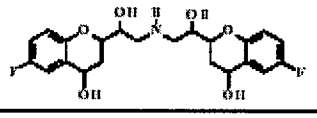
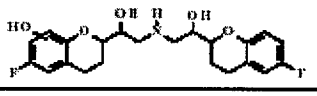
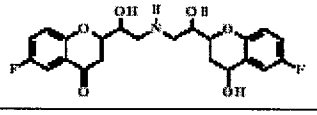
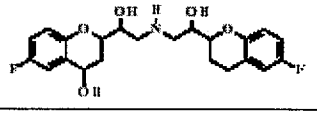
Metabolites Identified or Characterized following Incubation of a mixture of [^{14}C]-*d*- and [^{14}C]-*l*-Nebivolols with Liver Microsomes from Female Mice, Rats, Dogs, and Humans

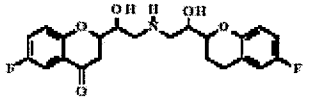
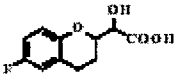
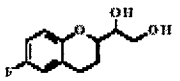
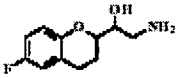
Test System	Substrate	A-6	A-8
	d	ND	Figure 25
	l	Figure 28	Figure 28
	d	ND	Figure 25
	l	ND	Figure 28
	d	Figure 25	Figure 25
	l	Figure 28	Figure 28
	d	Figure 25	Figure 25
	l	Figure 28	Figure 28

ND: Not Detectable

Appears This Way On Original

Nebivolol and Its Metabolites Characterized or Identified in the *in vitro* Systems

Met. Code No	Met Code Name	MW	Representative Structure
UD	Unchanged Drug (*d,l*-Nebivolol)	405	
A-1	Diphenol Nebivolols	437	
A-2	Phenolic Diol Nebivolols	453	
A-3	Phenolic Monohydroxy Nebivolols	437	
A-4	Phenolic Dehydro Nebivolols	419	
A-5	Diol Nebivolols	437	
A-6	Monophenol Nebivolols	421	
A-7	Keto Hydroxy Nebivolols	435	
A-8	Mono Hydroxy Nebivolols	421	

Met. Code No	Met Code Name	MW	Representative Structure
A-9	Mono Keto Nebivolols	419	
A-10	Dealkylated Carboxylic Acid	226	
A-11	Dealkylated diol	282	
A-12	Dealkylated hydroxy-amine	281	

XBLStudy # 04685, XBL report # RPT01174Search and Investigation of Nebivolol Metabolites in Plasma Samples from Human, Rat, Mouse, and Dog Using Liquid Chromatography-Tandem Mass Spectrometry (LC/MS/MS) techniques

Study location:
Study date: initiated August 2004
GLP: no
QA: no
Test article:

Compound	Lot Number	Purity
[^{14}C]-*d*-nebivolol:	X1505:19A	
[^{14}C]-*l*-nebivolol:	X1505:19B	
d-nebivolol:	S46730/1	
l-nebivolol:	S46731/1	

Plasma samples from the more recent pharmacokinetic studies were used for this analysis as detailed in the sponsor's table below. The samples were extracted followed by centrifugation. The extract was concentrated and analyzed by LC/MS or LC/MS/MS. The following metabolite groups were investigated :
4-OH nebivolols (A-8)
monophenolic nebivolols (A-6)
phenolic 4-OH nebivolols (A-3)
4-keto nebivolols (A-9)
diphenolic nebivolols(A-1)
N-dealkylated hydroxyl carboxylic acid (A-10)
Nebivolol (UD)
Glucuronic nebviolols (G-UD)

1 Page(s) Withheld

b Trade Secret / Confidential

_____ Draft Labeling

_____ Deliberative Process

Withheld Track Number: Pharm/Tox-_____

The 2 studies reviewed in this addendum are in vitro versus in vivo determinations of nebivolol metabolites. The results of the 2 studies are compared in the reviewer's table below:

Comparison of the in vitro and in vivo nebivolol metabolite determinations

In vitro	In vivo
Predominant metabolites: Monohydroxy nebivolols (A8) Monophenols (A-6) except in rodents Phenolic monohydroxy nebivolols(A-3) Diol nebivolols (A-5) Monoketo nebivolols (A-9)	Predominant metabolites in all tested species: Nebivolol glucuronides (G-UDs) Monophenol nebivolol glucuronides (G-6) Monohydroxy nebivolols (A-8) Monohydroxy nebivolol glucuronides (G-8) N-dealkylated carboxylic acid (A-10) Next most predominant: Diol nebivolol glucuronides (G-5) Keto hydroxyl nebivolol glucuronides (G-7) monoketo nebivolol glucuronides (G-9) N-dealkylated diol (A-11) N-dealkylated hydroxylamine (A-12)
Minor in all species: N-dealkylated carboxylic acid (A-10) N-dealkylated diol (A-11) Only in mouse and dog N-dealkylated hydroxyl amine (A-12)	

Unchanged drug which was a major component of the in vitro systems was not mentioned in the overall summary. As might be expected, there are differences between in vitro and in vivo results. The in vivo glucuronides of the in vitro hydroxyls and phenols are an expected modification.

This study was initiated several months into the NDA review and completed late in the NDA review cycle. The nature of the work is something that would have been of greater value at a much earlier stage in the development process. A glaring omission in the recent attempts to clarify PK and metabolites by application of LC/MS/MS is the lack of any studies examining PK and metabolism in gravid animals.

The material submitted does not substantially alter the overall non-clinical picture of nebivolol.

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Elizabeth Hausner
12/21/04 12:59:56 PM
PHARMACOLOGIST
Elizabeth Hausner

Albert Defelice
1/4/05 12:19:23 PM
PHARMACOLOGIST

PHARMACOLOGY/TOXICOLOGY REVIEW

3.1 INTRODUCTION AND DRUG HISTORY

NDA number: 21742
Review number: 1
Sequence number/date/type of submission: 0/November 23, 2004/
Information to sponsor: Yes ( ) No ( )
Sponsor and/or agent: Bertek
Manufacturer for drug substance: Mylan Pharmaceuticals Inc, 781 Chestnut Ridge Road, Morgantown, WV 26505.

Reviewer name: **Elizabeth Hausner, D.V.M.**
Division name: **Cardio-Renal Drug Products**
HFD #: 110
Review completion date: November 29, 2004

Drug:

Generic Name: Nebivolol Tablets

Chemical Name Nebivolol hydrochloride is identified chemically as (±)-[2R*[R*[R*(S*)]]]-α,α′-[iminobis(methylene)]bis[6-fluoro-3,4-dihydro-2H-1-benzopyran-2-methanol] hydrochloride

Code Numbers R067555

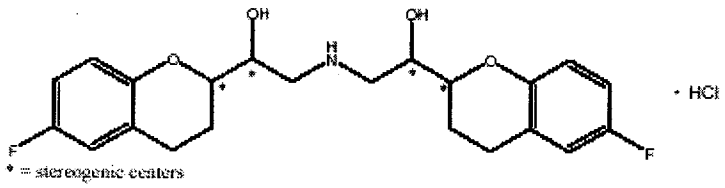
R067138 (d-Nebivolol)

R067145 (l-Nebivolol)

CAS Registry No.152520-56-4

Trade Name: To Be Established

Figure 3.2-01 Chemical Structure of Nebivolol



Empirical Formula $C_{22}H_{25}F_2NO_4 \cdot HCl$

Molecular Weight 441.90 g/mol

Due to 4 chiral carbons, there are 10 different stereoisomers possible. The drug substance is the racemate of the enantiomeric pair SRRR-nebivolol (d-nebivolol) and RSSS-nebivolol (l-nebivolol).

The present submission was presented in response to a telecom September, 2004. The sponsor was informed that the Exec CAC had determined the Leydig cell tumors in mice to be drug related. The sponsor was asked to make a case to show that this finding was not clinically relevant. Some weeks later the sponsor announced their intention of having the slides from the mouse carcinogenicity study re-evaluated. The Division in turn asked the sponsor to put into writing the rationale for re-evaluating the slides and to provide in writing the protocol that was proposed for the endeavor.

The sponsor did not provide the rationale nor a protocol. The sponsor presented the present material as a *fait accompli*. The slides were re-evaluated in a "blinded" fashion then a Pathology Working Group was assembled. In the PWG, all testes slides from all 62 animals originally identified as having an interstitial cell proliferative lesion were re-evaluated. Additional slides were randomly selected from 30 mice originally diagnosed as having no proliferative lesion. The testes slides for these 30 mice were used to provide the basis of the "normal" comparison.

The sponsor's results are shown below.

EXPERIMENT NO. 1967
CARCINOGENICITY STUDY IN SWISS MICE

Table 2
Incidence of Histomorphologic Observations in the Testes

Dose Group:	1	2	3	4	5
Dosages^:	C	V	L	M	H
Sex:	M	M	M	M	M
Number of Mice/Group:	50	50	50	50	50
TESTES:					
NO. EXAMINED	50	50	50	50	50
-negative for hyperplasia or neoplasia	44	43	46	44	23
-neoplasia, interstitial-cell, present	1	2	0	3	17[1]
-adenoma, interstitial-cell, unilateral, single	1	2	0	2	9
-adenoma, interstitial-cell, unilateral, multiple	0	0	0	1	1
-adenoma, interstitial-cell, bilateral, multiple	0	0	0	0	7
-carcinoma, interstitial-cell, unilateral, single	0	0	0	0	2
-hyperplasia, interstitial-cell, present	5	6	4	3	16
-hyperplasia, interstitial-cell, unilateral, focal	0	0	2	0	11
-hyperplasia, interstitial-cell, unilateral, multifocal	1	1	0	0	1
-hyperplasia, interstitial-cell, unilateral, diffuse	0	2	0	0	0
-hyperplasia, interstitial-cell, bilateral, multifocal	1	1	1	0	2
-hyperplasia, interstitial-cell, bilateral, diffuse	3	2	1	3	2

^Dosages: C: Control non-medicated diet
V: Vehicle β-cyclodextrin (β-CD) (440 mg/kg body weight/day)
L: Low 2.5 mg R 67555/kg body weight/day and 27.5 mg β-CD/kg body weight/day
M: Mid 10 mg R 67555/kg body weight/day and 110 mg β-CD/kg body weight/day
H: High 40 mg R 67555/kg body weight/day and 440 mg β-CD/kg body weight/day

[1]Neoplasia was observed in 17 mice of the high dose group, but there were 19 individual tumors. Two mice (#233 and #238) had both interstitial-cell adenoma and interstitial-cell carcinoma.

From the above table and the next table, it is unclear how many carcinomas are actually present. Is it 2 alone, or 2 alone and 2 in combination or 2 in combination? The text of the report states that there was a reclassification of 1 adenoma to a carcinoma. While there were a few re-classifications (in both directions), the overall interpretation did not change.

Table 2. Summary of Interstitial (Leydig) Cell Proliferative Lesions Incidence in 62 Mice Based on PWG Consensus Diagnoses

	Control	Vehicle	2.5 mg/kg/day	10 mg/kg/day	40 mg/kg/day
Number of mice in group	50	50	50	50	50
Mice with hyperplasia only	4	4	5	4	9
Mice with adenoma only	1	1	0	0	11
Mice with adenoma and carcinoma	0	0	0	0	1
Mice with hyperplasia and adenoma	0	1	0	1	4
Mice with adenoma or carcinoma	1	2	0	1	16
Mice with hyperplasia, adenoma or carcinoma	5	6	5	5	25

The sponsor also provided reprints of a number of published references:

R.F. McConnell et al.(1992).Proliferative lesions of the testes in rats with selected examples from mice, URG-3 In :Guides for Toxicologic Pathology. STP/ARP/AFIP, Washington, D.C.

K. Mitsumori and M.R. Elwell (1988). Proliferative lesions in the male reproductive system of F344 rats and B6C3F1 mice: incidence and classification. Environ Health Pers. Vol77, pp.11-21.

F.K. Mostofi and V.M. Bresler. Tumors of the testis. Year and location of publication unknown.

S. Rehm et. al. Male Genital System. Year and location of publication unknown.

The reprints were descriptions of the histologic features of hyperplasia and various tumor types that may be found in the testes and reproductive tract of male mice and rats.

The overall result of this effort did not change the conclusion of the tumorigenic potential of the drug. The material presented does not address the Division's question as to the relevance or lack of relevance for humans.

Elizabeth Hausner, DVM
Pharmacologist

Al DeFelice, PhD
Supervisory Pharmacologist

Appears This Way
On Original

This is a representation of an electronic record that was signed electronically and this page is the manifestation of the electronic signature.

/s/

Elizabeth Hausner
12/21/04 01:02:20 PM
PHARMACOLOGIST
Elizabeth Hausner

Albert Defelice
12/30/04 10:57:05 AM
PHARMACOLOGIST