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assigned to placebo. Thirteen of the subjects given placebo, were also taking an ACE inhibitor.

**Study population:**

Sixty subjects aged 18 years or older, who were diagnosed with New York Heart Association (NYHA) Class II, III or IV CHF.

Among the 29 recipients of Natrecor and ACE inhibitors, 19 subjects were taking enalapril, 4 subjects were taking lisinopril, 4 subjects were taking captopril, one subject was taking ramipril and one subject was taking quinapril.

**Design:**

Randomized, double-blind, placebo-controlled Phase II dose-response study.

Blood was collected at the following time points: 15 minutes prior to dosing, 2, 5, 15 minutes, 1 and 1.5 hours after the first and last dose.

**Assay procedures:**

Please refer to Appendix 9 for details about the assay. The methodology used was an ELISA and was described previously. Briefly,

The LOQ was      pg/ml. Quality control samples were not provided.

The mean concentration of endogenous hBNP is reported to be highly variable amongst subjects and it is also reported to be variable within a subject. To adjust for the presence of endogenous hBNP, the pretreatment concentration of hBNP was subtracted from hBNP concentrations measured in post-dosing samples.

**Data analysis:**

Only the first dose data of ACE inhibitor recipients in this study was used in the analysis. The parameter estimates for the 19 enalapril recipients were compared with those for the first dose data of the 42

subjects in study 704.309. individual comparisons involving the other ACE inhibitors were not done.

The PK parameters were measured using model-independent methods. The reason for this choice of data analysis is reported to be the lack of enough concentration-time points for a two-compartment model. The values of C<sub>max</sub> were normalized to a dose of 5 µg/kg.

### Results:

The mean parameter estimates for subjects given Natreacor plus enalapril and Natreacor plus any ACE inhibitor is presented in the following table (Table 1):

Table 1

Mean Estimates of Pharmacokinetic Parameters

	hBNP Plus Enalapril (n = 19)	95% Confidence Interval	hBNP Plus Any ACEI (n = 29)	95% Confidence Interval	hBNP without ACEI (Study 704.309, n = 42)	95% Confidence Interval
C <sub>max</sub> (ng/mL)	33.4	18.6, 48.2	34.6	33.8, 45.3	36.5	29.6, 43.3
CL (mL/min/kg)	9.89	7.99, 11.80	9.75	8.25, 11.25	9.8	7.8, 11.8
V <sub>area</sub> (L/kg)	0.26	0.20, 0.33	0.27	0.22, 0.32	0.24	0.20, 0.29
V <sub>ss</sub> (L/kg)	0.24	0.18, 0.30	0.25	0.18, 0.24	0.21	0.18, 0.24
k (h <sup>-1</sup> )	2.39	2.19, 2.60	2.27	2.11, 2.44	2.53	2.27, 2.78
t <sub>1/2</sub> (h)	0.30	0.27, 0.33	0.32	0.29, 0.34	0.30	0.27, 0.32
MRT (h)	0.40	0.35, 0.44	0.41	0.37, 0.45	0.38	0.34, 0.41

The parameters listed above are reported not to differ significantly from those reported in the 42 subjects in study 704.309 given only Natreacor (please refer to Appendix 2).

The following is the mean difference of each parameter without enalapril versus with enalapril, including the 95% confidence intervals:

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CL:	0.12 ml/min/kg (-3.10, 3.34 ml/min/kg)
Varea:	0.02 L/kg (-0.07, 0.10 L/kg)
Vss:	0.03 L/kg (-0.03, 0.09 L/kg)
MRT:	0.02 hours (-0.04, 0.08 hours)
Parameter k:	-0.13 h <sup>-1</sup> (-0.53, 0.27 h <sup>-1</sup> )
Half-life:	0.00 hours (-0.04, 0.04 hours)
Cmax:	-3.1 ng/ml (-3.10, 3.34 ng/ml)

**Conclusions:**

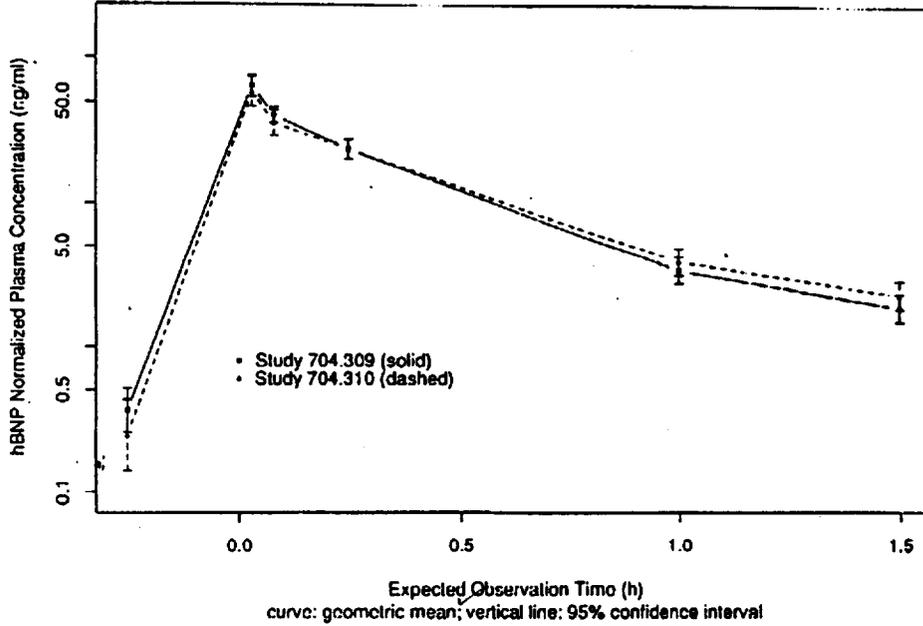
The sponsor reports that the present study was conducted because of reports of possible effects of ACE on the plasma elimination kinetics of porcine BNP on rats. In that report, captopril increased the half-life of porcine BNP in rats from 1.23 minutes to 6.99 minutes, and captopril also increased the steady state concentration of porcine BNP from a continuous IV infusion. The sponsor reports that the structure of Natrecor is different from that of porcine BNP.

In the present study, Natrecor was administered as an intermittent intravenous bolus at doses of 3, 5 and 10 µg/kg every 4 hours for 24 hours to subjects with congestive heart failure who were also taking ACE inhibitors (specifically enalapril). The following parameters were determined:

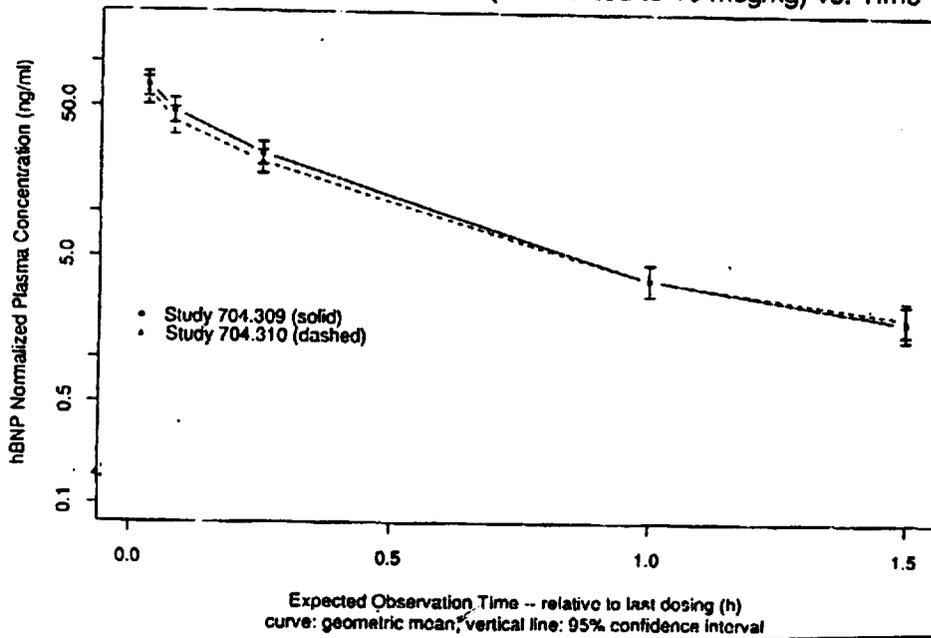
CL:	9.89 ml/min/kg (7.99, 11.80 ml/min/kg)
Varea:	0.26 L/kg (0.20, 0.33 L/kg)
Vss:	0.24 L/kg (0.18, 0.30 L/kg)
MRT:	0.40 hours (0.27, 0.33 hours)
Parameter k:	2.39 h <sup>-1</sup> (2.19, 0.26 h <sup>-1</sup> )
Half-life:	0.30 hours (0.27, 0.33 hours)
Cmax:	33.4 ng/ml (18.6, 48.2 ng/ml)

The following 2 figures show hBNP plasma concentration vs time profiles after the first and last doses of an intermittent bolus dosing regimen with an without enalapril. The solid line shows the data from study 704.309 where no enalapril was used (and which was described in Appendix 2) and the dashed line shows the results from the present study (704.310) where enalapril was coadministered to the subjects. No difference is seen in the blood levels of hBNP with or without enalapril.

Scios Study 704.309 and 704.310 (all first dose data)  
hBNP Plasma Concentration (normalized to 10 mcg/kg) vs. Time



Scios Study 704.309 and 704.310 (all last dose data)  
hBNP Plasma Concentration (normalized to 10 mcg/kg) vs. Time



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**Appendix 4**

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**Pharmacokinetic analysis of plasma concentrations of Natrecor hBNP from study 704.306 (IND( ) "A phase II randomized, double-blind, placebo-controlled, ascending dose-response study of a hemodynamic, renal and neurohormonal effects of the continuing infusion of Natrecor hBNP in subjects with congestive heart failure"**

**Study No. 704.306**  
**Report No. DAD 96-03a**

**Volume 1.32**

**Pages 2-82**

Report date: November 3<sup>rd</sup>, 1997 (revised)

**PK data analyzed by:** Nancy Sambol, PharmD (UCSF)  
Romain Sechaud, Ph.D.

**Objectives:**

To assess the drug's safety, hemodynamic, renal and neurohormonal effects; and pharmacokinetics at two dose levels, according to Scios Inc. clinical protocol 704.306.

**Medication:**

Natrecor: 5 mg lyophilized powder reconstituted in 5% dextrose in water (lot # E00013A1). The Natrecor was produced by synthetic peptide methodology.

Placebo: 5% dextrose in water.

**Dose level:**

Continuous fixed-dose IV infusion of Natrecor for 4 hours:

0.025 µg/kg/min (n=6)

0.05 µg/kg/min (n=6)

placebo (n=4)

This was an ascending dose study, therefore the 0.025 µg/kg/min dose was complete (including 2 placebo subjects), prior to initiation of infusion of 0.05 µg/kg/min (with the other 2 placebo subjects).

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**Study population:**

Sixteen subjects aged 18 years or older, who were diagnosed with New York Heart Association (NYHA) Class III CHF.

**Design:**

Randomized, double-blind, placebo-controlled Phase I/II dose-response study.

Blood was collected at the following time points: -4, 0, 0.17, 0.5, 1.5, 3, 4, 4.08, 4.17, 4.5, 5, 6, and 7 hours (relative to start of infusion).

**Assay procedures:**

The methodology used was an ELISA and is described in Appendix 9. Briefly,

was      pg/ml. Quality control samples were not provided.      The LOQ

**Data analysis:**

The mean endogenous hBNP was highly variable between subjects and somewhat variable within each subject. This was determined from the placebo group. Human BNP plasma concentrations were modeled (1 compartment open model) using      as the sum of concentration from the infused and estimated endogenous hBNP concentration. The estimated endogenous hBNP was modeled as a constant, using values from before and after the infusion. The sponsor notes that this approach recognizes that variability in the endogenous concentrations of BNP exist. Weighted least squares regression analysis was used for the fitting, with a weight of  $y^{-1}$ .

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**Results:**

The following table describes the PK parameters calculated in this study. The endogenous hBNP levels were estimated from the mean value before infusion and after the elimination of the infused dose. The observed values for the endogenous hBNP levels were determined from the baseline determinations. These values showed significant variability among subjects, as noted from the first column in the table. Additionally, there seemed to be some variation in the clearance values among subjects within the same group, even after these values had been normalized for body weight. The average calculated PK parameters are as follows:

For the 0.025 µg/kg/min dose:

$$\begin{aligned} \text{CL} &= 9.7 \pm 1.5 \text{ ml/min/kg} \\ \text{Vd} &= 0.26 \pm 0.04 \text{ L/kg} \\ \text{T}_{1/2} &= 26 \pm 6 \text{ min} \end{aligned}$$

For the 0.05 µg/kg/min dose:

$$\begin{aligned} \text{CL} &= 17.3 \pm 3.1 \text{ ml/min/kg} \\ \text{Vd} &= 0.26 \pm 0.05 \text{ L/kg} \\ \text{T}_{1/2} &= 12 \pm 2 \text{ min} \end{aligned}$$

Interestingly, the clearance of the drug seemed to be increased in the higher dose group, although the sponsor attributes this to an outlier (subject 324-012) who had a clearance of 31 ml/min/kg. Consequently, the  $t_{1/2}$  is reported to be lower in the higher dose group. The mean difference in CL for the two dose groups was 7.62 mL/min/kg. The sponsor states that this difference approached significance with a p value of 0.0509.

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**Table 1**  
**Pharmacokinetic Parameter Estimates of hBNP in Subjects**  
**with Congestive Heart Failure after a Continuous Intravenous Infusion**

Subject ID	Endogenous hBNP Concentration		CL		V <sub>d</sub>		k <sub>10</sub>	t <sub>1/2</sub>
	Observed <sup>1</sup> (pg/mL)	Estimated (pg/mL)	(mL/min)	(mL/min/kg)	(L)	(L/kg)	(min <sup>-1</sup> )	(min)
<b>Dose:</b> 0.025 µg/kg/min								
324-002	140	123	851	10.8	27	0.34	0.032	22
324-003	2969	2964	316	15.6	21	0.27	0.015	46
324-004	926 <sup>2</sup>	855	475	6.9	28	0.34	0.017	41
324-005	1553	1332	752	6.4	10	0.15	0.073	10
324-006	227	212	1285	11.3	38	0.33	0.034	21
324-007	2462	2405	456	7.3	9	0.14	0.052	13
Mean:	1313	1315	689	9.7	22	0.26	0.037	26
SE:	476	475	144	1.5	4.6	0.04	0.009	6
<b>Dose:</b> 0.050 µg/kg/min								
324-009	705	644	1032	11.6	20	0.22	0.052	13
324-011	3415	3510	929	10.5	18	0.20	0.050	14
324-012	1326	1370	2224	31.1	12	0.17	0.180	4
324-013	951	1013	1284	19.2	32	0.48	0.040	17
324-014	1542	1663	1068	18.4	16	0.28	0.066	11
324-015	585	656	1033	13.2	15	0.19	0.070	10
Mean:	1421	1476	1262	17.3	19	0.26	0.076	12
SE:	426	438	198	3.1	2.9	0.05	0.021	2
Overall Mean:	1367	1396	975	13.5	21	0.26	0.057	19
Overall SE:	305	309	145	2.0	2.6	0.03	0.013	4

<sup>1</sup> Observed at baseline.

<sup>2</sup> Only one predose concentration available (otherwise there were two).

**Conclusion:**

This study was aimed at determining the PK profile of Natrecor following IV infusion for 4 hours to subjects with CHF. The doses used in the study were 0.025 and 0.05  $\mu\text{g}/\text{kg}$ . The CL values determined were  $9.7 \pm 1.5$  ml/min/kg (mean  $\pm$  SE) for the 0.025  $\mu\text{g}/\text{kg}$  dose and  $17.3 \pm 3.1$  ml/min/kg (mean  $\pm$  SE). The difference between these clearances approached significance ( $p=0.0509$ ). The sponsor states that the presence of an outlier in the 0.050  $\mu\text{g}/\text{kg}$  group did not permit drawing the conclusion that in fact the two treatment groups tended to have different clearances. However, the reviewer calculated the median for the clearance values in both dose groups and these were not very different from the means (9.05 and 15.8, for the low and high dose group medians, respectively). This indicates that the outlier is not solely responsible for the difference in clearances observed between the 2 dose groups. It should also be noted that the sample size was rather small ( $n=6$ ). The overall CL for the 2 treatment groups was  $13.5 \pm 2.0$  ml/min/kg. The  $V_d$  was not different between the 2 dose groups, however the  $t_{1/2}$  values for the low dose group tended to be higher than those for the higher dose group. The sponsor does not report the statistical comparison of this parameter ( $t_{1/2}$ ) between the groups.

The steady state plasma concentrations reached in the individual subjects have not been reported other than in graphical format. These values were calculated by the reviewer and are reported as follows:

0.025  $\mu\text{g}/\text{kg}$  Natrecor:  $2847 \pm 927$  pg/ml (mean  $\pm$  SD)  
0.05  $\mu\text{g}/\text{kg}$  Natrecor:  $3298 \pm 1190$  pg/ml

In conclusion, following a 4-hour infusion of Natrecor, the average  $t_{1/2}$  is  $19 \pm 4$  minutes (mean  $\pm$  SE) and the average clearance is  $13.5 \pm 2.0$  ml/min/kg. The average  $V_d$  is  $0.26 \pm 0.03$  L/kg. A large amount of variability among subjects was noted in endogenous plasma levels of hBNP, as well as in the clearance of exogenous hBNP.

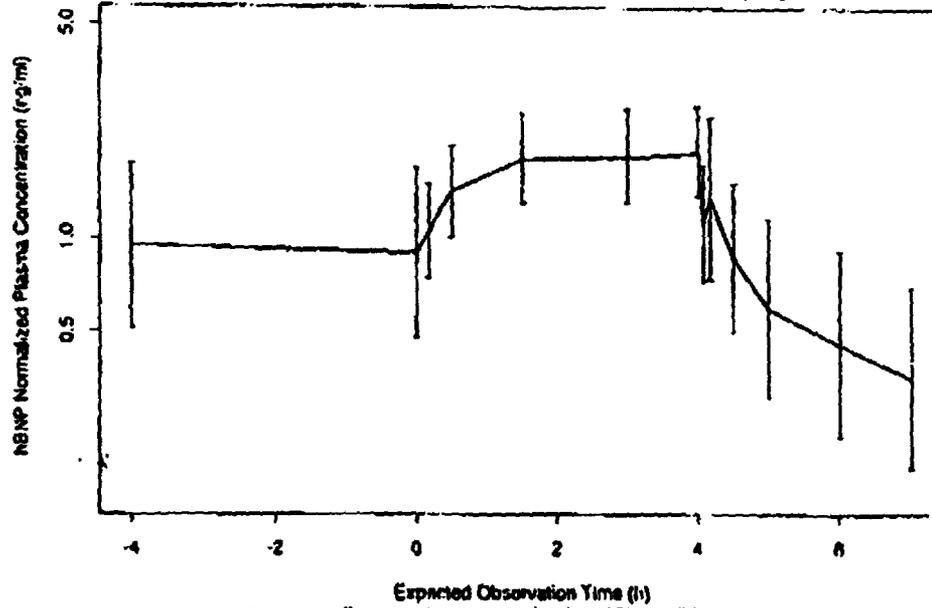
The following 2 figures show the hBNP plasma concentration vs time profiles for the 4-hour continuous infusion study described above. The first figure shows the results which are dose-normalized to the 0.015  $\mu\text{g}/\text{kg}/\text{min}$  dose, whereas the second figure shows the data dose-normalized to the 0.025  $\mu\text{g}/\text{kg}/\text{min}$  dose.

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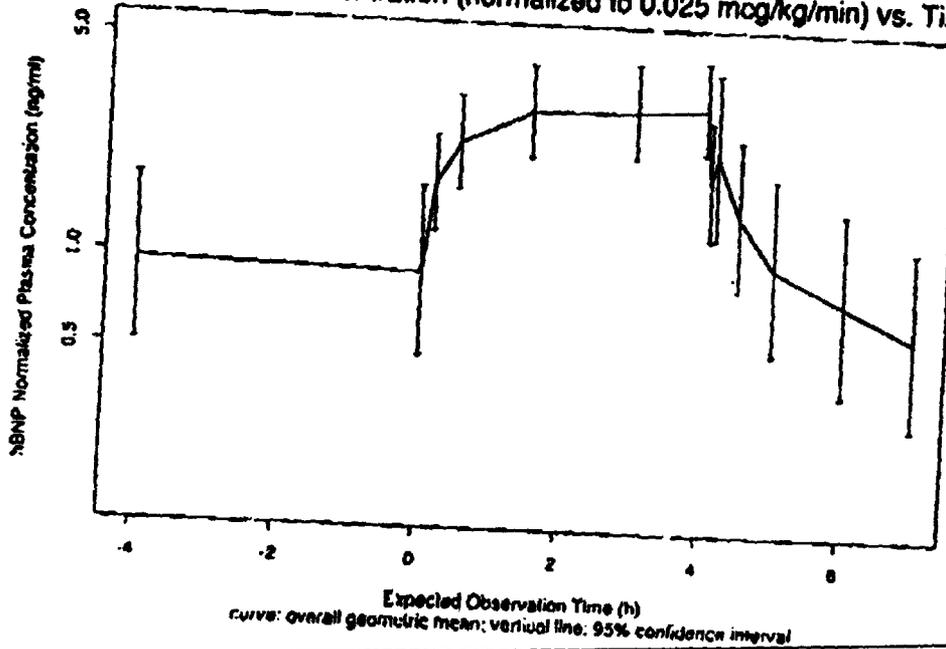
Natrecor  
Nesiritide  
03/25/99

Scios, Inc.

Scios Study 704.306  
hBNP Plasma Concentration (normalized to 0.015 mcg/kg/min) vs. Time



Scios Study 704.306  
hBNP Plasma Concentration (normalized to 0.025 mcg/kg/min) vs. Time



Appendix 5

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**Pharmacokinetic-pharmacodynamic analysis of Natrecor hBNP from study 704.307 (IND ) "A phase II randomized, double-blind, placebo-controlled, crossover study of the hemodynamic effects of an intravenous incremental dose infusion of Natrecor hBNP in subjects with congestive heart failure"**

**Study No. 704.307**  
**Report No. DAD 95-03**

**Volume 1.32**

**Pages 83-211**

Report date: January 7<sup>th</sup>, 1998 (revised)

**Investigators:** Milton Packer, MD.  
Columbia University

**PK data analyzed by:** Nancy Sambol, PharmD (UCSF)  
Helen Kastrissios, Ph.D.  
Chui Yu Liu

**Objectives:**

1. To evaluate the linearity in the pharmacokinetics of Natrecor hBNP over the dose range studied.
2. To characterize the relationships between changes in PCWP (pulmonary capillary wedge pressure), SVR (systemic vascular resistance) and cardiac index (CI) responses and steady state plasma concentrations of infused hBNP.
3. To examine the effects of various patient characteristics on the pharmacokinetics and pharmacodynamics of hBNP, using a population analysis approach.

**Medication:**

Natrecor: 5 mg lyophilized powder reconstituted in 5% dextrose in water (lot # E00013A1). The Natrecor was produced by synthetic peptide methodology.

Placebo: 5% dextrose in water.

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**Dose level:**

IV incremental-dose infusion of Natrecor hBNP at 0.003, 0.01, 0.03, or 0.1 µg/kg/min in ascending order. The doses were increased every 1.5 hours.

**Study population:**

Twenty subjects aged 18 years or older, who were diagnosed with New York Heart Association (NYHA) Class II, III or IV CHF. All subjects were hospitalized for 3 days.

**Design:**

Randomized, double-blind, placebo-controlled Phase II incremental dose infusion study. Subjects were randomized to receive either placebo on day 1 and Natrecor on day 2, or Natrecor on day 1 and placebo on 2. An infusion pump was used to administer a continuous intravenous infusion of Natrecor or placebo, through a peripheral IV line. The infusion began at a rate of 0.003 µg/kg/min and was incrementally increased to 0.01 µg/kg/min and then to 0.03 µg/kg/min at 1.5 hour intervals. After 1.5 hours, the dose was increased to 0.1 µg/kg/min, however 7 subjects were maintained at the 0.03 µg/kg/min dose for 3 hours.

Hemodynamic measurements (PCWP, SVR and CO) were performed at 1, 1.5, 2.5, 3, 4, 4.5, 5.5 and 6 hours (1 and 1.5 hours after each dose escalation) and 7 hours (1 hour post infusion).

Blood was collected at the following time points: preinfusion, 1.5, 3, 4.5, (after 1.5 hours of infusion at each dose level, immediately before dose escalation), 6 hours (at infusion completion), and 7 hours (1 hour postinfusion).

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**Assay procedures:**

The methodology used was an ELISA that is described in Appendix 9. Briefly,

The LOQ was      pg/ml.

**Data analysis:**

Data not collected at steady state were excluded from the analysis, because the sponsor states that they were insufficient to permit the necessary model to be fit to them. Therefore, all single point postinfusion plasma hBNP levels and pharmacodynamic measurements were not analyzed.

It is stated that pharmacokinetic-pharmacodynamic analyses were undertaken in a modular approach, in order to take into account the contribution of endogenous hBNP concentrations and baseline pharmacodynamics and to avoid confounding these effects with those of Natrecor.

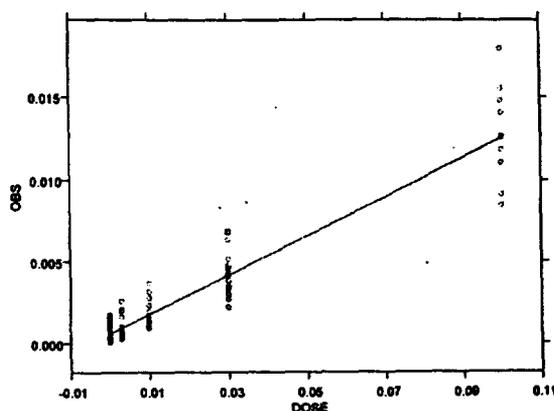
A pharmacokinetic model that accounts for the endogenous hBNP was developed. The predicted concentrations from the pharmacokinetic model were then introduced as independent variables in the pharmacodynamic model. The pharmacodynamic endpoints (PCWP, SVR and CI) were analyzed independently and were tested for effects from the administration of placebo. The physiological activity of endogenous hBNP was tested in the pharmacodynamic models at the various stages of analysis (i.e., analysis of placebo/baseline effect and drug effect). The influences of patient covariates on the pharmacokinetics (clearance) and pharmacodynamics (baseline effect and parameter of drug effect) of Natrecor were also tested. All analyses were performed using NONMEM.

**Results:**

The following results were reviewed and analyzed by Dr. Raymond Miller.

**Pharmacokinetics**

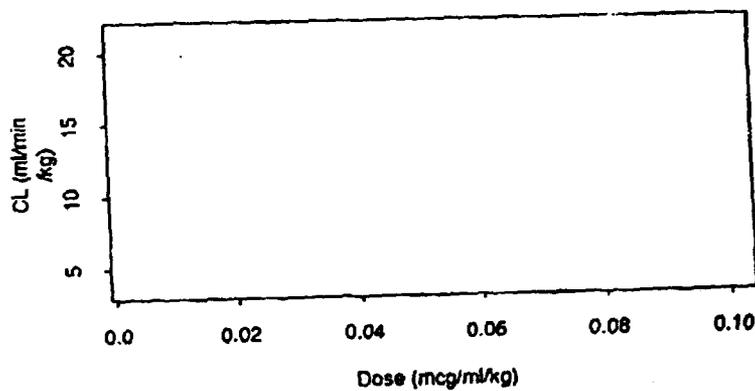
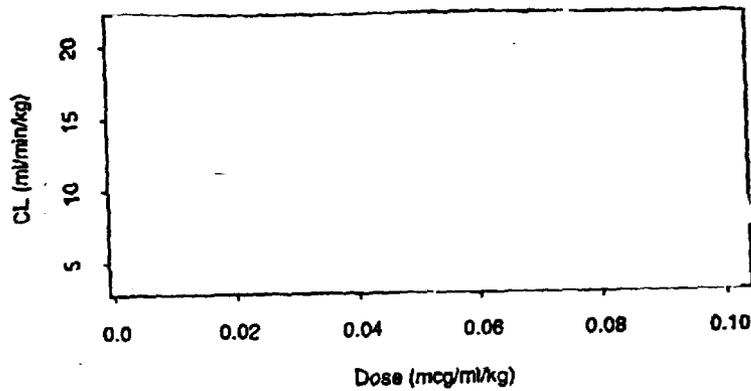
A model for the plasma clearance (CL) of hBNP was developed that takes into account subject-specific endogenous plasma hBNP concentrations. The mean plasma CL of hBNP was estimated to be 9.2 mL/min/kg with a 95% confidence interval of 8.4 to 10.1 mL/min/kg. Individual clearance estimates covaried among doses. The lowest dose had a significantly



larger interindividual variability in CL (47%, compared to 21% to 31% in the higher doses). Therefore, a stabilization transformation was used to align the variability at the low dose with that associated with all the other doses to minimize bias that may be carried forward in the pharmacodynamic analyses. The following figures illustrate the distribution of individual hBNP CL estimates at each dose before (top panel) and after the stabilization transformation (bottom panel).

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Distribution of Individual hBNP CL Estimates at each Dose  
(top panel) and After Imposing a Stabilization Transformation (bottom panel)



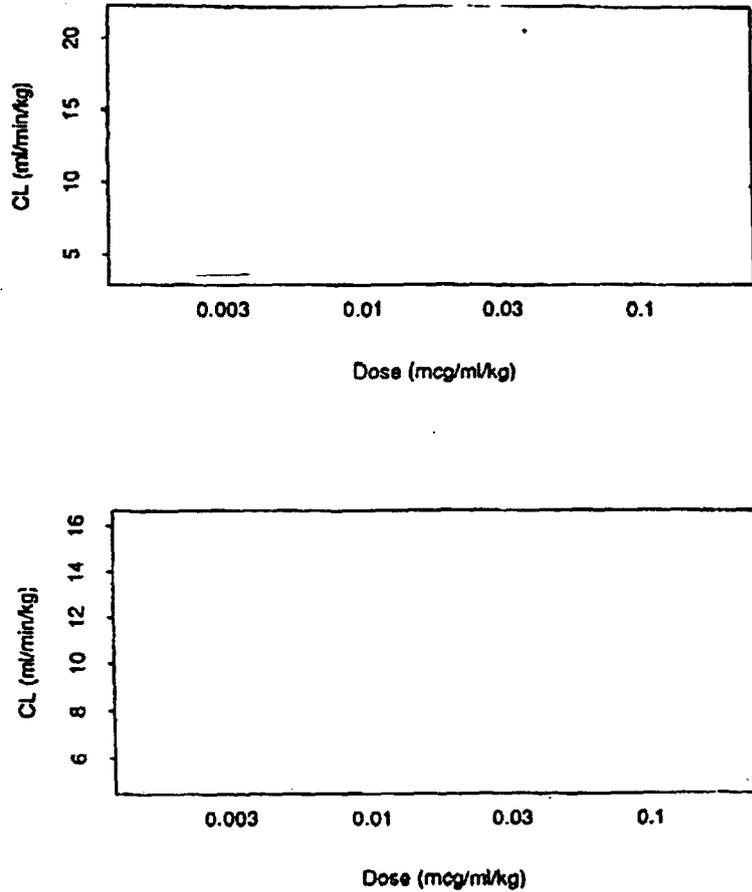
Dotted lines connect individual observations (i.e. ID).

The following figures illustrate the same observations as above, using a box and whisker plot:

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Figure 4b

Distribution of Individual hBNP CL Estimates at each Dose  
(top panel) and After Imposing a Stabilization Transformation (bottom panel)



Horizontal line within box is median, box bounds the interquartile range (IQR = 1st-3rd quartiles), dashed lines connect 1.5 x IQR, outer horizontal lines are outliers.

There was generally a strong correlation of CL within individuals from dose to dose. There was no suggestion of nonlinear pharmacokinetics over the dose range studied. A scatterplot of observed concentration versus dose supports this claim.

A summary of the population PK parameter estimates is listed in the following table:

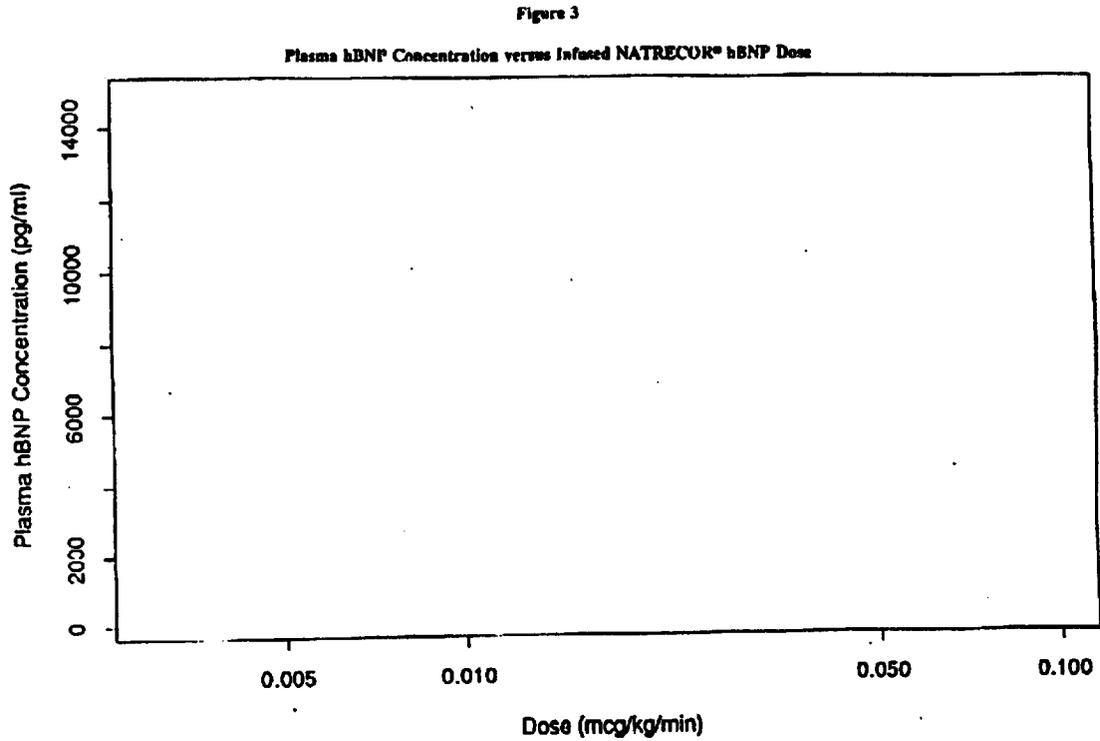
Table 1

## Summary of Population Pharmacokinetic Parameter Estimates

Parameter	Mean	95% Confidence Interval
Endogenous hBNP concentration (pg/ml)	596	413, 779
Clearance (CL) (mL/min/kg)	9.2	8.4, 10.1
Intraindividual variability (% CV) in CL		
0.003 µg/kg/min	47.0	19.4, 63.6
0.01 µg/kg/min	30.9	12.7, 41.8
0.03 µg/kg/min	20.7	8.6, 28.0
0.1 µg/kg/min	24.8	8.8, 33.9
Correlations between doses		
0.003 and 0.01 µg/kg/min	0.99	
0.003 and 0.03 µg/kg/min	0.96	
0.01 and 0.03 µg/kg/min	0.95	
0.003 and 0.1 µg/kg/min	0.25	
0.01 and 0.1 µg/kg/min	0.18	
Intraindividual variability (%CV) in endogenous plasma hBNP concentration	80.0	51.2, 100.9
Residual variability (%CV)	11.9	9.2, 14.2

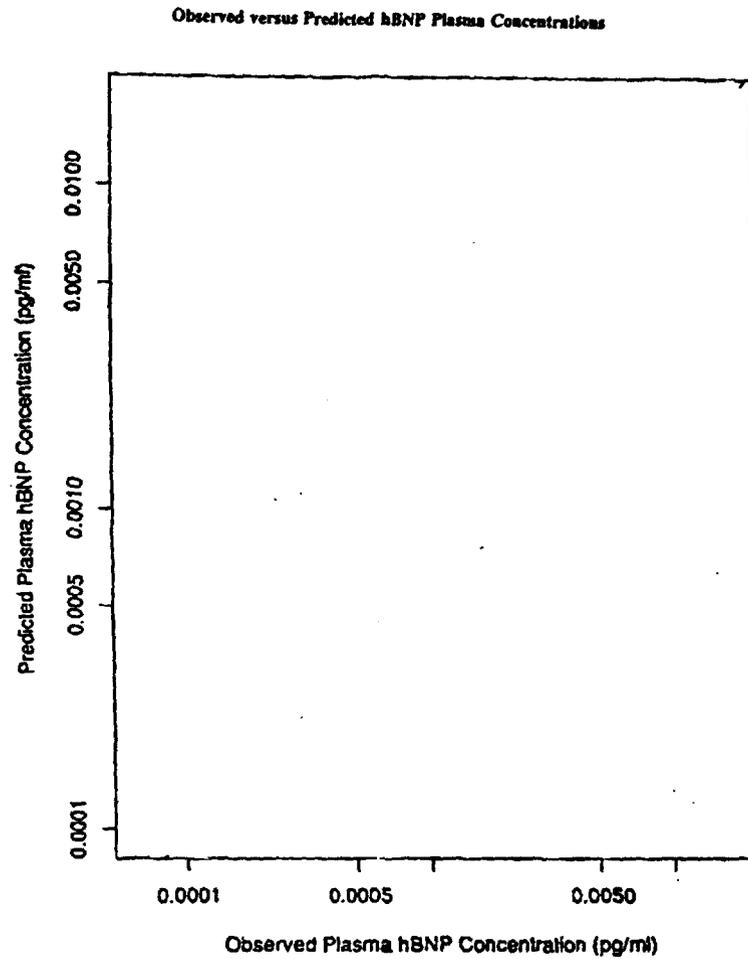
Plasma hBNP concentrations versus infused Natreacor dose is shown in the following figure:

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The following figure shows the relationship between observed and predicted steady-state plasma hBNP concentration, with imposing the stabilization transformation versus without imposing the stabilization transformation.

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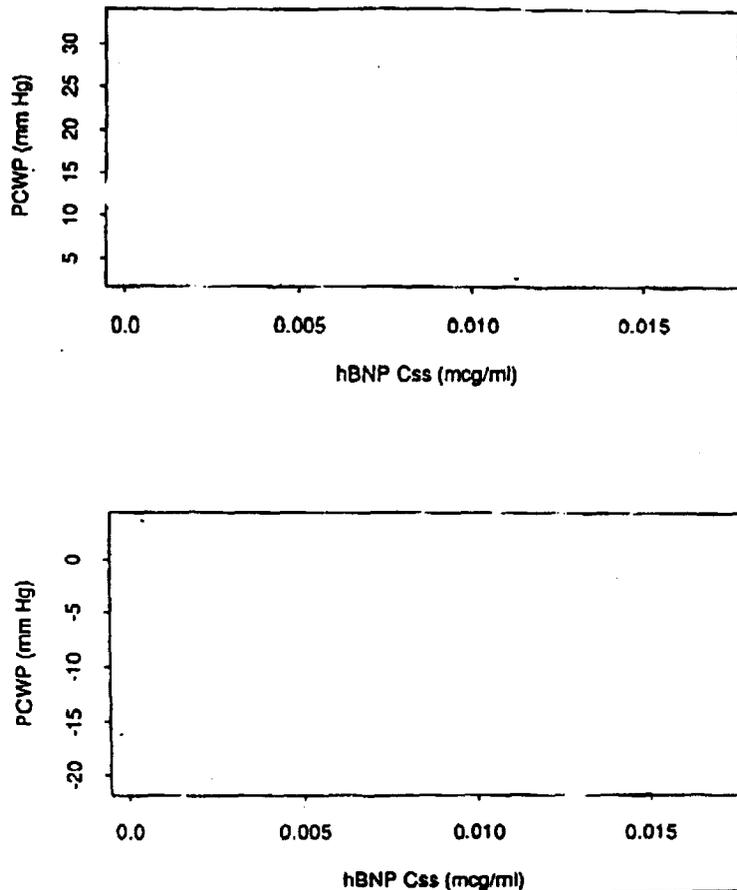
Solid line is unity,  $n = 113$ .

### Pharmacodynamics

Three hemodynamic responses, pulmonary capillary wedge pressure (PCWP), cardiac index (CI), and systemic vascular resistance (SVR) were analyzed.

The following figures show the steady-state plasma hBNP concentration versus PCWP (figure 7a), CI (figure 7b) and SVR (figure 7c).

Steady-State Plasma hBNP Concentration versus PCWP  
(top panel) and Change in PCWP from Baseline (bottom panel)



The dotted lines connect the individual observations. C<sub>ss</sub> is adjusted for endogenous concentration.

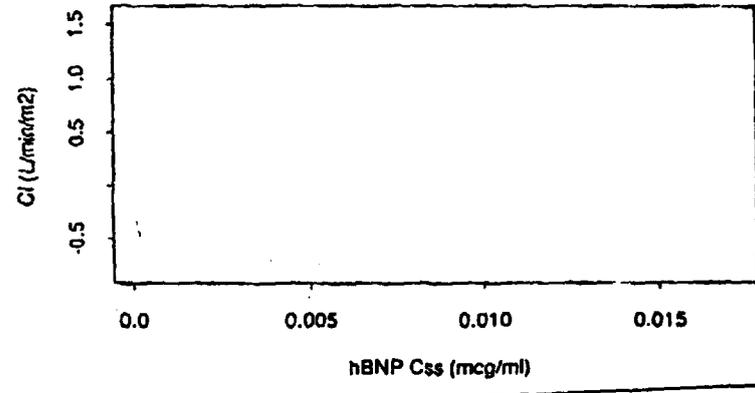
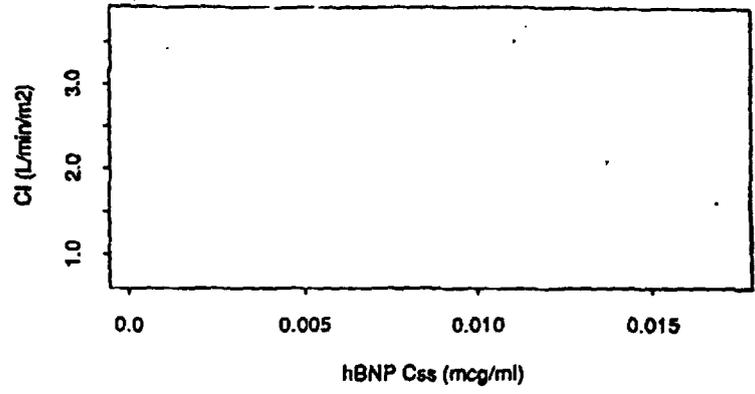
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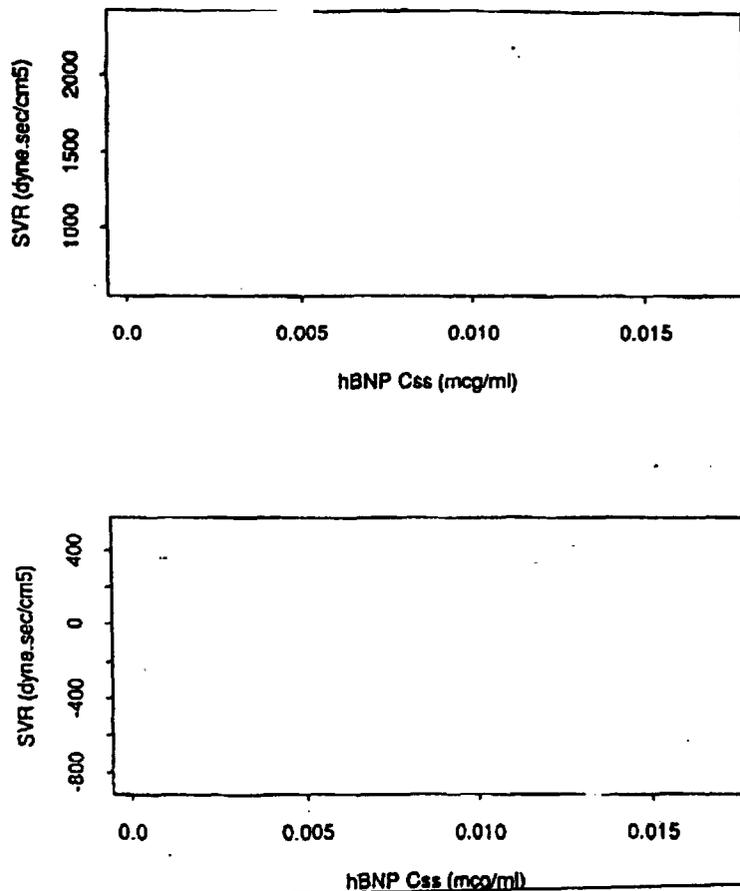
Steady-State Plasma hBNP Concentration versus CI  
(top panel) and Change in CI from Baseline (bottom panel)



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Figure 7c

Steady-State Plasma hBNP Concentration versus SVR  
 (top panel) and Change in SVR from Baseline (bottom panel)



A saturation model with sigmoidicity (sigmoid Emax model) was determined to best describe the relationship between (predicted) steady-state exogenous plasma hBNP concentration and each hemodynamic response tested.

$$E = E_{base} + \frac{E_{max} \cdot C_{SSk}^{\gamma}}{C_{SSk}^{\gamma} + C_{50}^{\gamma}}$$

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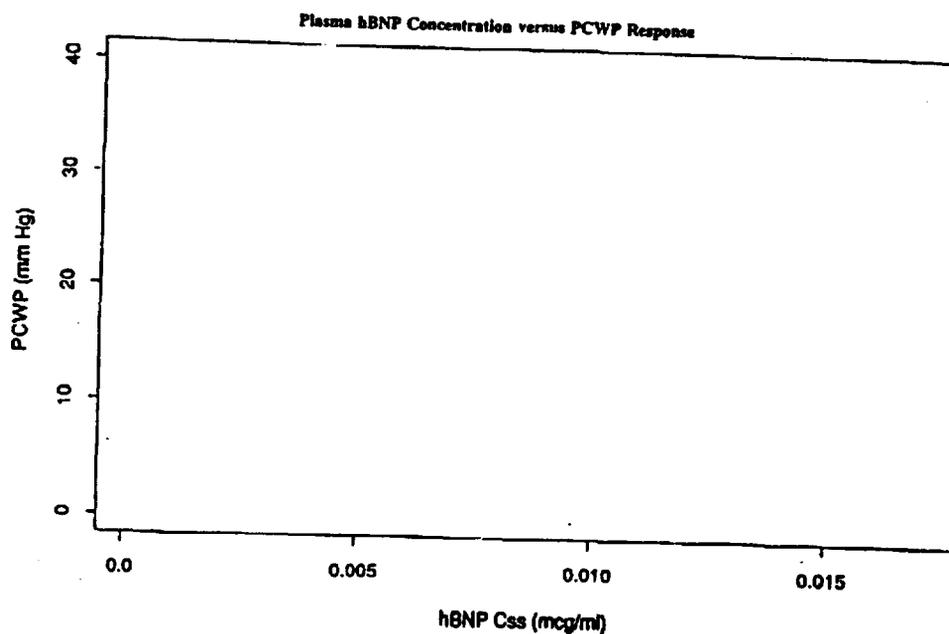
in which E is the effect, Ebase is the subject-specific estimate of baseline effect obtained previously, Emax is the maximum effect attributable to the drug, C<sub>ss</sub> is the predicted plasma hBNP concentration after the k<sup>th</sup> dose, C<sub>50</sub> is the plasma hBNP concentration that produces 50% of the maximum effect, and  $\gamma$  is the sigmoidicity factor that describes the steepness of the concentration versus response relationship. An Emax model ( $\gamma = 1$ ) was also tested.

There was no evidence of a placebo response for any of these hemodynamic endpoints. Pharmacodynamic parameters were assumed to be log-normally distributed, and residual variability was reasonably constant for each of the responses.

The maximum reduction in PCWP in subjects with NYHA classification II and III CHF during infusion of NATRECOR® hBNP was estimated to be 16.2 mm Hg (95% confidence interval: 13.6 mm Hg, 18.8 mm Hg). The 2 subjects with severe CHF (NYHA class IV) demonstrated a blunted response, estimated at 42% (95% confidence interval: 22%, 62%) of the value of the other two groups. During the stepwise deletion of covariates, only the effect of disease severity on Emax was significant. The following graph shows the plasma hBNP concentration at steady-state versus the PCWP response as well as the line for the predicted relationship between plasma hBNP and PCWP decrease.

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Figure 8



x = indiv observations in NYHA Class II & III, o = indiv observations in NYHA Class IV, solid line = predicted concentration-response curve (Class II & III), dashed line = predicted concentration response curve (Class IV)

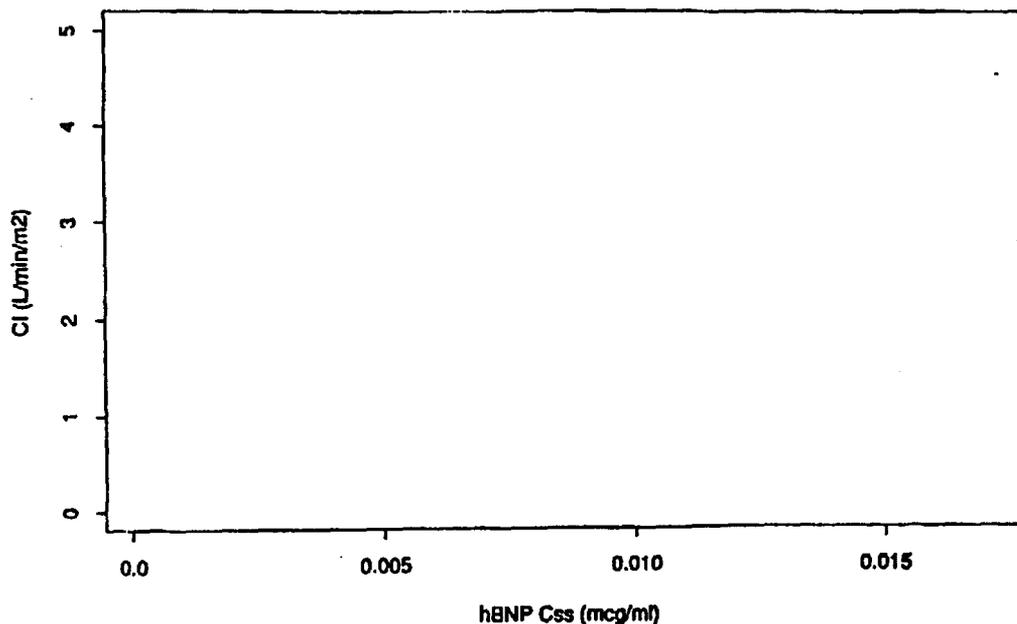
Maximum changes in CI and SVR in response to a given concentration of hBNP varied with subject body weight.

The predicted plasma hBNP concentration-CI response curve is shown in the following figure:

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Figure 9

Plasma hBNP Concentration versus CI Response

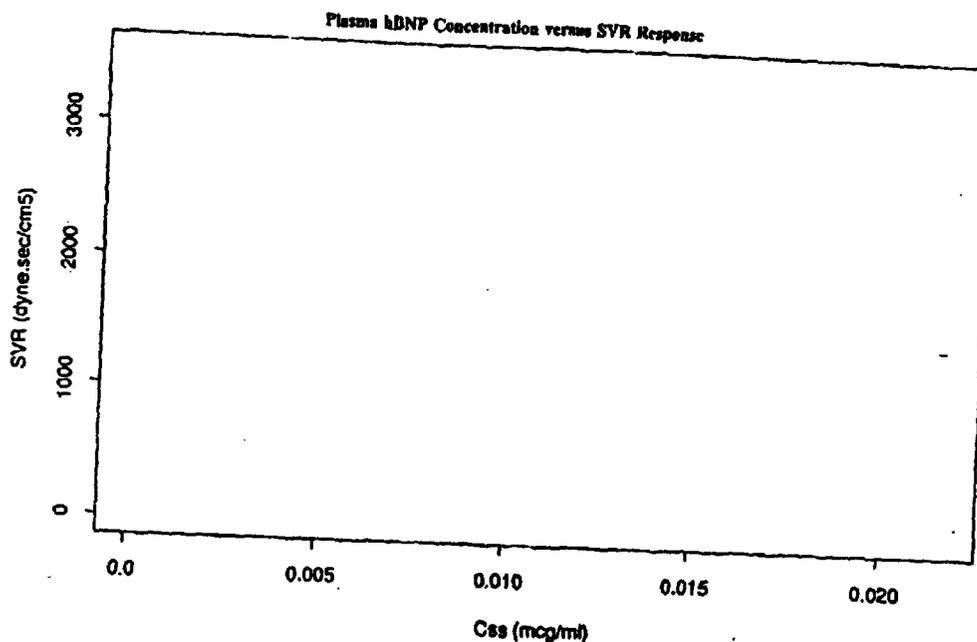


x = individual observations, solid line = predicted concentration-response curve.

The maximum increase in CI was estimated to be 0.68 L/min/m<sup>2</sup> (95% confidence interval: 0.27 L/min/m<sup>2</sup>, 1.08 L/min/m<sup>2</sup>) and the maximum reduction in SVR 450 dynes.sec.cm<sup>-5</sup> (95% confidence interval: 180 dynes.sec.cm<sup>-5</sup>, 720 dynes.sec.cm<sup>-5</sup>) for the subject who has a median weight (80.5 kg). For both responses, there was a mean change (loss of drug effect with larger individuals) of approximately 8% to 9% for each kilogram that a subject's weight differed from 80.5 kg.

The following graph shows the relationship between the predicted plasma hBNP concentration versus SVR. The dashed lines represent the predicted values for heavier and lighter individuals.

Figure 11



x = individual observations, solid line = predicted concentration response curve for median weight subject (80.5 kg), dashed line for 70.5-kg subject, long short dashed line for 90.5-kg subject.

The plasma hBNP concentration that produces 50% of the maximal reduction, C<sub>50</sub>, in PCWP response was estimated to be 2400 pg/mL (95% confidence interval: 1500 pg/mL, 3300 pg/mL), which corresponds approximately to a steady-state infusion of 0.02 µg/min/kg (assuming the mean CL). At the same time, C<sub>50</sub> for SVR was also estimated to be 2400 pg/mL (95% confidence interval: 500 pg/mL, 4300 pg/mL), whereas a slightly higher C<sub>50</sub> of 3100 pg/mL (95% confidence interval: 700 pg/mL, 5500 pg/mL) was estimated for CI.

### Conclusions

The estimated mean endogenous plasma hBNP concentration of 596 pg/mL is consistent with that reported to be found in moderate to severe CHF (Class II through IV). Possible mechanisms for this elevation in subjects with CHF is a reduction in hBNP CL and/or an increased

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cardiac production of hBNP. The present results support a mechanism based on a reduced hBNP clearance, because the estimated mean hBNP CL of 9.2 mL/min/kg is approximately one-seventh of that reported in healthy subjects after infusion of similar doses of hBNP. The results of the current analysis suggest hBNP clearance is greater in patients who weigh more.

Left ventricular function improved during infusions of hBNP, as indicated by observable reductions in both PCWP and SVR and an increase in CI. Relationships between hBNP concentration and each of the hemodynamic endpoints could be described simply using a sigmoid E<sub>max</sub> (saturation) model. There was no apparent delay in response after plasma hBNP concentrations had reached a steady-state, and therefore a more complex model incorporating an effect compartment was not required. Additional support for this finding came from analysis of hemodynamic responses at early (after 1 and 1.5 hours) and delayed (after 2.5 and 3.0 hours) times following escalation to the 0.03 µg/min/kg dose level in 6 subjects. There was no significant difference in responses measured between early and late times, suggesting that, in addition to plasma hBNP concentrations, the pharmacodynamics had also achieved equilibrium.

All baseline hemodynamic measurements in this study were similar to those observed in other studies of subjects with moderate to severe CHF. The mean C50 for both PCWP and SVR was estimated to be 2400 pg/mL. Assuming mean hBNP clearance, a steady-state concentration approximately equal to the C50 may be expected after continuous infusion of 0.02 µg/min/kg. Based on the mean estimates of E<sub>max</sub> for PCWP of -16 mm Hg in patients with Class II or III CHF and for SVR of -450 dynes.sec.cm<sup>-5</sup>, this infusion rate is predicted to decrease PCWP by about 8 mm Hg and SVR (in the typical patient weighing 80.5 kg) by about 225 dynes.sec.cm<sup>-5</sup>. Individuals weighing considerably more or less than 80.5 kg are predicted to require a dosage adjustment to achieve the same SVR. A slightly higher C50 of 3100 pg/mL (corresponding to an infusion rate of 0.03 µg/min/kg) was estimated for CI. Administration of this dose to a patient of typical weight would be expected to increase CI by 0.34 L/min/m<sup>2</sup>.

### **Reviewers Comment**

The sponsor asserts that there is no apparent delay in response after plasma hBNP concentrations has reached a steady-state. While the

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observations do not allow for visual inspection of this feature an alternative model can be tested against the currently proposed PK/PD model. A hypothetical biophase steady-state concentration is used to relate concentration to effect as:

$$C_e^{ss} = C_p^{ss} \cdot (1 - \exp^{-k_{e0}t})$$

where,  $k_{e0}$  represents the dissociation constant of the drug-receptor binding. Determining the  $k_{e0}$  allows the appreciation of the onset/offset properties of the PD effect. The sponsors analysis of hemodynamic responses at early (after 1 and 1.5 hours) and delayed (after 2.5 and 3.0 hours) times following escalation to the 0.03  $\mu\text{g}/\text{min}/\text{kg}$  dose level in 6 subjects showing no significant difference in responses measured between early and late times, suggests that the pharmacodynamics has achieved equilibrium after one hour of constant infusion.

It is clear that exogenous doses of hBNP show beneficial therapeutic effects in the management of CHF. The concentration-response relationship was shown to be graded, suggesting that the dose can be individualized within the range 0.003 to 0,1  $\mu\text{g}/\text{min}/\text{kg}$ . Endogenous levels of hBNP are raised in CHF and mediate compensatory mechanisms in the regulation of cardiovascular homeostasis and therefore, it may be worthwhile to model the baseline hBNP with a sigmoid Emax model as well as the exogenous hBNP. It may be assumed that the potencies of endogenous and exogenous hBNP are the same or different.

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**Appendix 6**

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**Pharmacokinetic analysis of Natrecor hBNP from study 704.311 (IND [redacted] A phase II randomized, double-blind, placebo-controlled, multicentre, dose-ranging study to evaluate the safety and efficacy of a 24 hour infusion of Natrecor hBNP in subjects with congestive heart failure"**

**Study No. 704.311**  
**Report No. DAD 96-03d**

**Volume 1.32**

**Pages 212-360**

Report date: February 2<sup>nd</sup>, 1998 (revised)

**PK data analyzed by:** Nancy Sambol, PharmD (UCSF)  
Romain Sechaud, Ph.D.  
Chui Yu Liu

**Objectives:**

To determine the PK of hBNP at more than one dose level when it was administered as an intravenous bolus followed by a 24-hour infusion.

**Medication:**

Natrecor: 5 mg lyophilized powder reconstituted in 5% dextrose in water (lot # E00013A1, G0003A1 and G0004A1). The Natrecor was produced by synthetic peptide methodology.

Placebo: 5% dextrose in water.

**Dose level:**

The following dose levels were used:

Placebo (n=29)

Natrecor 0.25 µg/kg IV bolus followed by 0.015 µg/kg/min IV infusion for 24 hours (n=21).

Natrecor 0.50 µg/kg IV bolus followed by 0.030 µg/kg/min IV infusion for 24 hours (n=27).

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Natrecor 1.0 µg/kg IV bolus followed by 0.060 µg/kg/min IV infusion for 24 hours (n=26).

The infusion was allowed to be decreased to 50% of the initial infusion rate if a subject experienced decreased blood pressure or PCWP. The lowest allowed dose by the protocol was 0.0075 µg/kg/min.

**Study population:**

One hundred and three subjects aged 18 years or older, who were diagnosed with New York Heart Association (NYHA) Class II, III or IV CHF.

**Design:**

The study was a randomized, double-blind, placebo-controlled, multicenter, dose-ranging study.

PK sampling of plasma was at the following time points: 15 minutes and immediately before dosing, 15 and 30 minutes and 1, 3, 6, and 24 hours after the administration of study drug was initiated. Additional samples were collected at 2, 5, 15, 30 and 60 minutes and 2 and 4 hours after the completion of drug infusion.

**Assay procedures:**

The methodology used was an ELISA that is described in Appendix 9. Briefly,

was      pg/ml.

The LOQ

**Data analysis:**

Only the steady state concentrations were analyzed. The analysis was conducted with a mixed effects model using maximum likelihood estimation and implemented with the NONMEM program. With this analysis, data are pooled and an overall model fit to the pooled data. The predicted concentration was modeled as the sum of the concentration attributable to the endogenous hBNP concentration (a constant) and that attributable to the infused hBNP.

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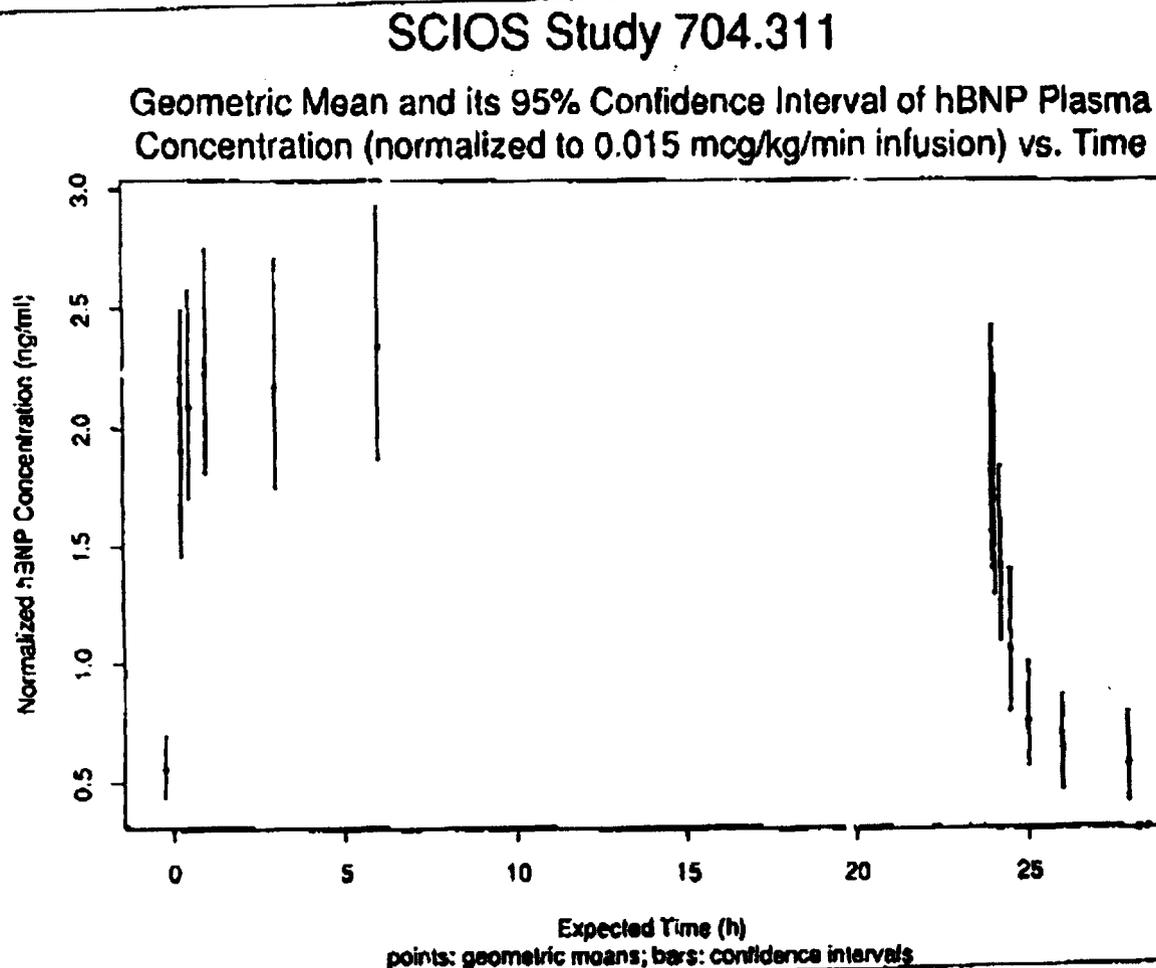
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Scios, Inc.

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**Results:**

The following graph shows the blood levels of hBNP in subjects for study 704.311:



The following section was reviewed, analyzed and written by Dr. Raymond Miller.

Clearance was found to be linearly correlated with body weight ( $p < 0.001$ ) and was estimated (without the outliers) to be 9.5 mL/min/kg (95% confidence interval: 7.9 mL/min/kg, 11.1 mL/min/kg).

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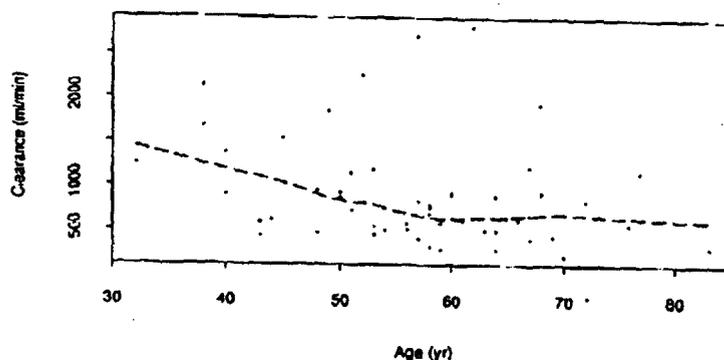
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Age, serum creatinine, baseline pulmonary capillary wedge pressure (PCWP), baseline cardiac index (CI), NATRECOR ® hBNP infusion rate, duration of NATRECOR ® hBNP administration, gender, race/ethnicity, and New York Heart Association CHF classification had no statistically significant (defined as  $p < 0.01$  in the final stage of testing) influence on clearance.

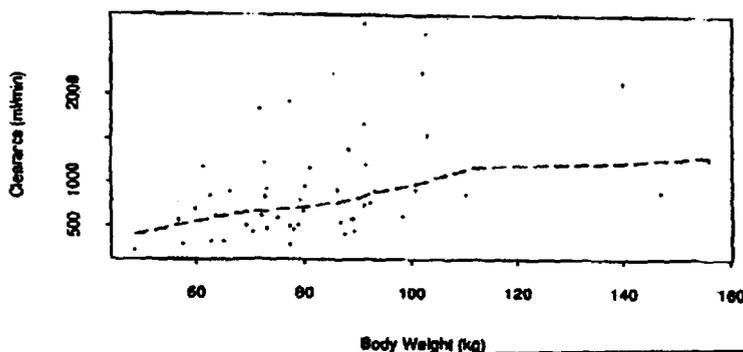
The following figures show the relationship between clearance and age, body weight, serum creatinine, creatinine clearance, baseline cardiac index, infusion rate, gender, ethnicity and New York Heart Association Classification:

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Figure 7a  
Posterior Estimates of hBNP Clearance vs. Age and Body Weight  
Clearance vs. Age



Clearance vs. Body Weight



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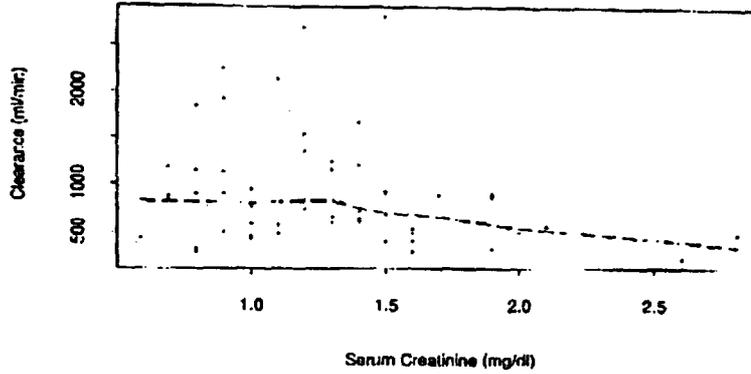
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The points represent the individual posterior estimates whereas the dashed line represents the mean curve.

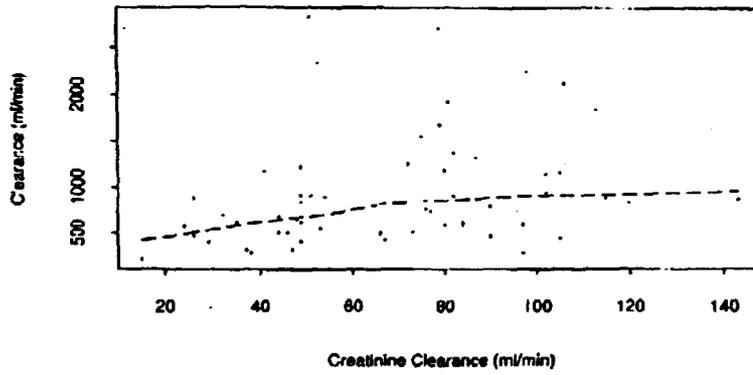
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Posterior Estimates of hBNP Clearance vs. Serum Creatinine and Creatinine Clearance

Clearance vs. Serum Creatinine



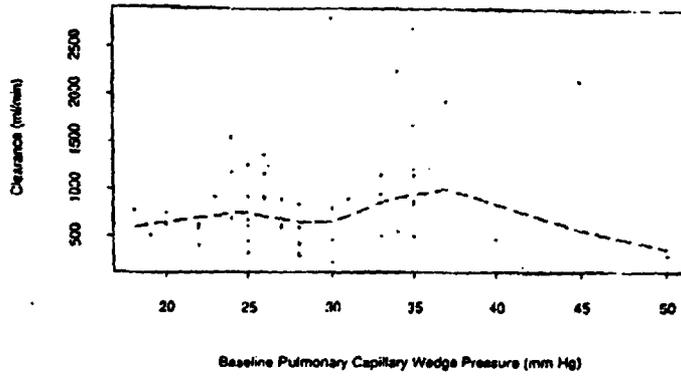
Clearance vs. Creatinine Clearance



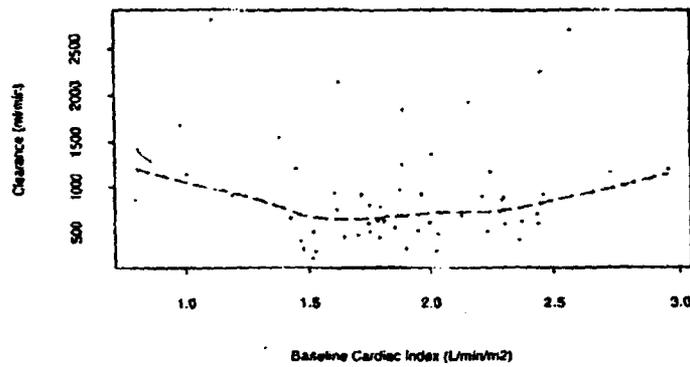
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Figure 16  
Posterior Estimates of hBNP Clearance vs.  
Baseline Pulmonary Capillary Wedge Pressure and Baseline Cardiac Index

Clearance vs. Baseline Pulmonary Capillary Wedge Pressure



Clearance vs. Baseline Cardiac Index

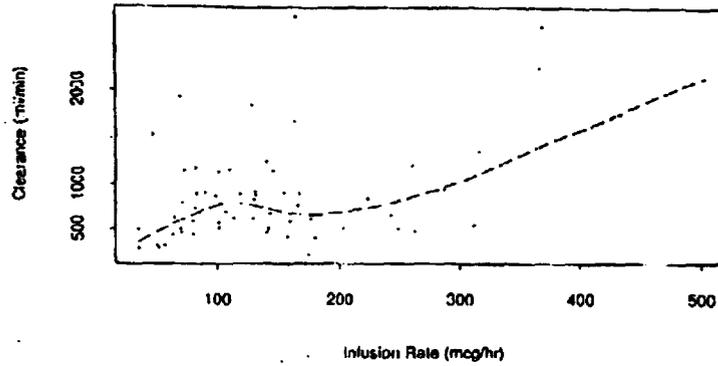


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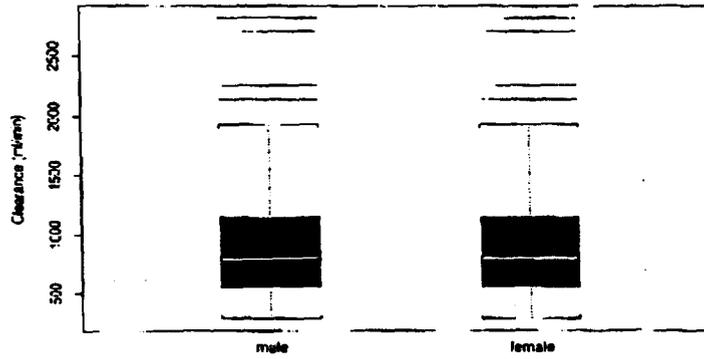
Figure 7d

Posterior Estimates of hBNP Clearance vs. NATRECOR® hBNP Infusion Rate and Gender

Clearance vs. NATRECOR hBNP Infusion Rate



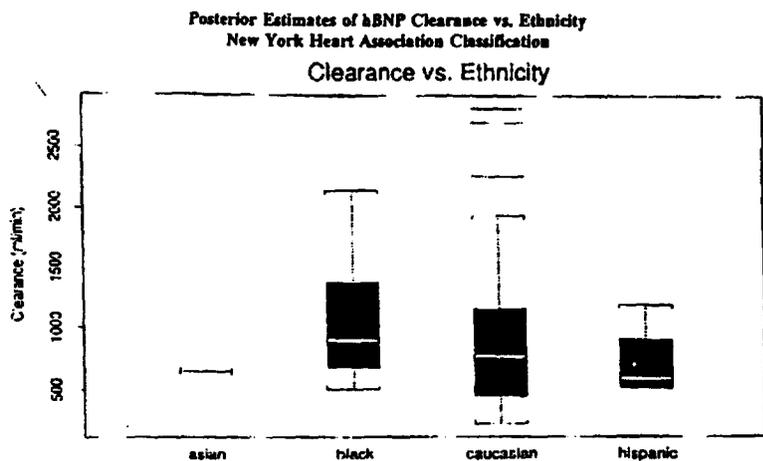
Clearance vs. Gender



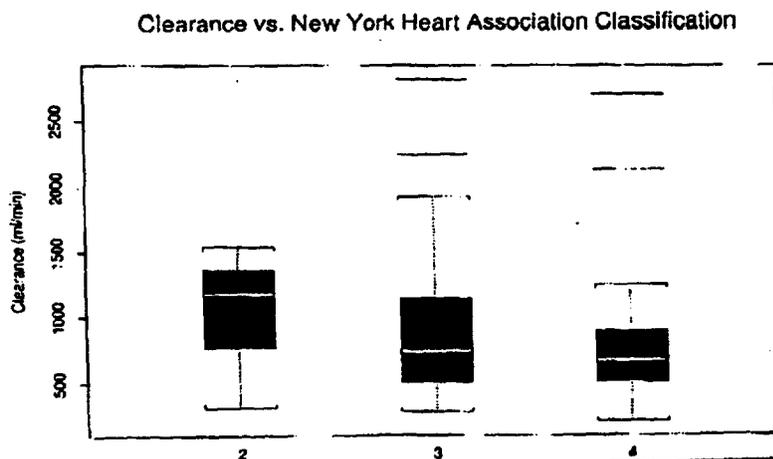
Gender  
central light bar: median; black box: middle 50%; square parentheses: range; black box: outlier

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Figure 7e



Ethnicity  
central light bar: median; black box: middle 50%; squared parentheses: range; black bar: outlier



The central bar represents the median; the black box represents the middle 50%; the squared parentheses represent the range and the black bar represents the outlier.

Creatinine clearance was a significant covariate for clearance only when the model for clearance did not include other covariates, particularly body weight. Although creatinine clearance was not included in the final model for hBNP clearance, due to the confounding nature of (calculated) creatinine clearance and weight, and the fairly limited range of creatinine clearance in this population (15 subjects had values of 30 to 49 mL/min and 5 had values < 30 mL/min), the possibility that renal function influences hBNP clearance cannot be ruled out. For these data, there is an approximately 99% chance that hBNP clearance decreases no more

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than approximately 10.9% with each 10 mL/min decrease in creatinine clearance.

In the final model:

- Clearance was found to be linearly correlated with body weight ( $p < 0.001$ ) and was estimated (without the outliers) to be 9.5 mL/min/kg (95% confidence interval: 7.9 mL/min/kg, 11.1 mL/min/kg).
- interindividual variability of CL was estimated to be 55% (95% confidence interval: 42%, 65%).
- endogenous hBNP plasma concentration was estimated to be 610 pg/mL (95% confidence interval: 443 pg/mL, 777 pg/mL)
- interindividual variability of the endogenous hBNP concentration was estimated to be 90% (95% confidence interval: 71%, 107%)
- The intraindividual and residual variability was estimated to be 26% (95% confidence interval: 20%, 31%).

**Reviewer comment**

The clearance of 9.5 ml/min/kg is consistent with values determined in other patients with a similar degree of congestive heart failure. Weight is the most important covariate for clearance, however, the possibility that kidney function influences hBNP CL cannot be ruled out. Weight and creatinine clearance are confounded and the range of creatinine clearance in this study is limited.

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Appendix 7

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**Pharmacokinetic analysis of Natrecor hBNP from study 704.325 (IND [redacted]) "A randomized, double-blind, placebo-controlled, study of two doses of Natrecor hBNP administered as a continuous infusion in subjects with decompensated congestive heart failure"**

**Study No. 704.325**  
**Report No. 00285**

**Volume 1.33**

**Pages 1-57**

Report date: February 18<sup>th</sup>, 1998 (revised)

**PK data analyzed by:** Nancy Sambol, PharmD (UCSF)  
Chui Yu Liu

**Objectives:**

To evaluate the safety and efficacy of IV Natrecor hBNP at 2 doses in the short-term management of decompensated CHF.

**Medication:**

Natrecor: 5 mg lyophilized powder reconstituted in 5% dextrose in water (lot # G0004A1 produced by synthetic peptide methodology and H0003A1 produced by recombinant DNA technology).

Placebo: 5% dextrose in water.

**Dose level:**

The following dose levels were used:

Placebo (n=42)

Natrecor 0.3 µg/kg IV bolus followed by 0.015 µg/kg/min IV infusion (n=43).

Natrecor 0.6 µg/kg IV bolus followed by 0.030 µg/kg/min IV infusion (n=42).

The treatment evaluation was carried out for 24 hours.

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The infusion was allowed to be decreased to 50% of the initial infusion rate if a subject experienced decreased systolic blood pressure below 85 mm Hg.

**Study population:**

One hundred and twenty seven subjects aged 18 years or older, who were diagnosed with CHF.

**Design:**

The study was a randomized, double-blind, placebo-controlled, multicenter, dose-ranging study.

PK sampling of plasma was at the following time points: 15 minutes before dosing and 6 and 24 hours after the initiation of study drug administration.

**Assay procedures:**

The methodology used was an ELISA that is described in Appendix 9. Briefly,

was pg/ml.

The LOQ

**Data analysis:**

The raw data included baseline, 6- and 24-hour hBNP plasma concentrations at steady state, and demographic and clinical variables for 69 subjects administered Natreacor.

Steady state is defined as the concentration achieved after the subject had received a constant rate of Natreacor infusion for 2 hours prior to sampling of blood. In this study, steady state is determined at the 6 hour sampling time.

The evaluation of the relationship between plasma clearance and demographic and physiological variables was based on the 6-hour

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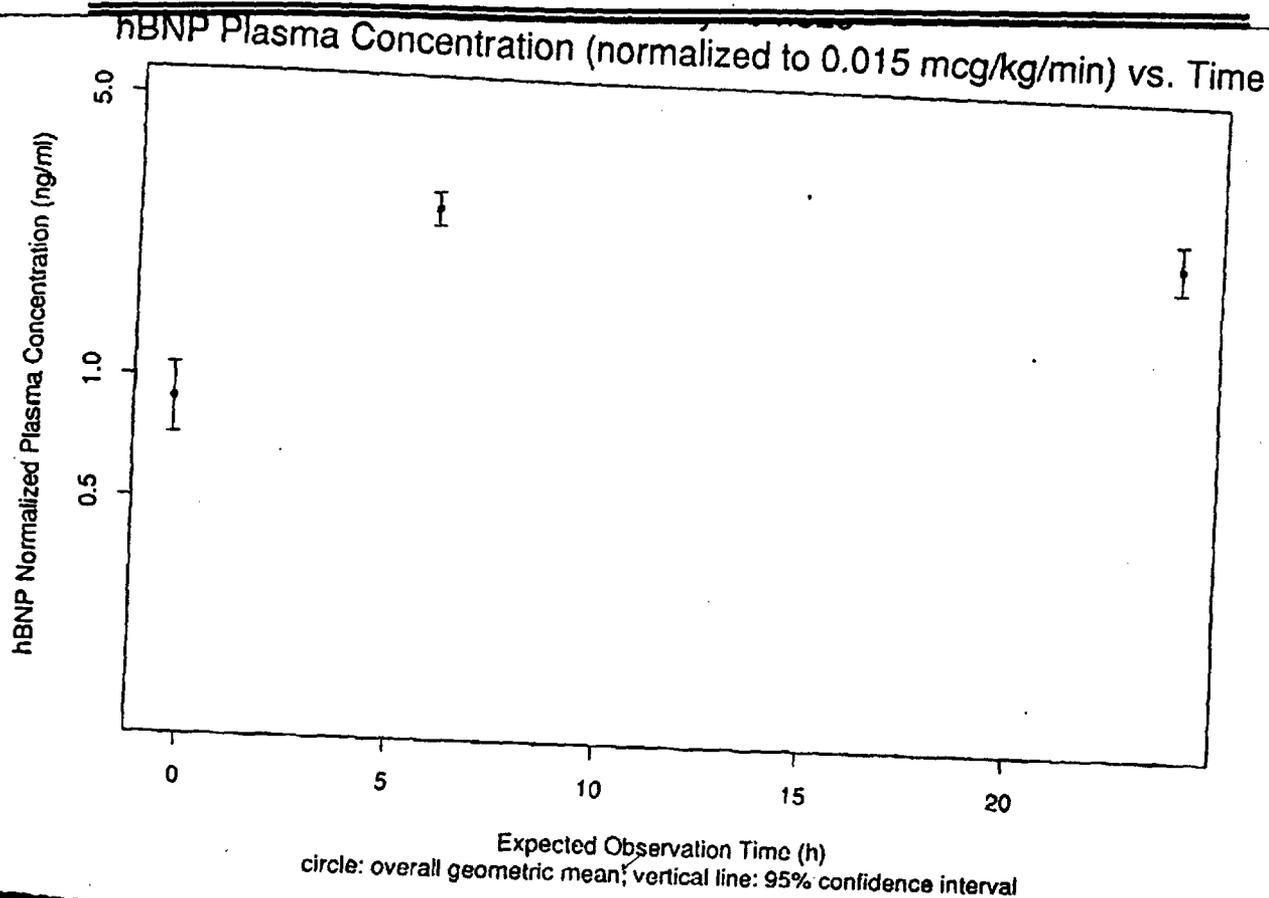
clearance data (n=65). For the evaluation of the duration of infusion on CL, data from subjects with constant infusion rates and evaluable CL values at 6 and 24 hours were compared by a paired Student's t-test with a 5% significance criterion (n=29). Data from subjects whose dose was reduced because of hypotension were excluded from the analysis. At the 24-hour time point, only 29 subjects were evaluable out of the initial 85 subjects dosed with Natrecor, due to missing plasma hBNp values, drug discontinuation or intercurrent dose modifications.

A nonparametric method was used to estimate the value of clearance ( $CL=R/C_{ss}-C_0$ , where  $C_0$  is the baseline level of hBNP). The dependence of CL on body weight, creatinine clearance, serum creatinine, age, baseline cardiac index and PCWP was investigated using linear regression with the null hypothesis being that the slope is zero. The relationship of CL and the severity of disease, ethnicity, gender, and method of hBNP production were investigated using the General Linear Models procedure with Student-Newman-Keuls test (SAS). If the 95% confidence interval of the slope excluded zero, the null hypothesis was rejected and the correlation deemed statistically significant.

**Results:**

The following figure shows the dose-normalized plasma levels of hBNP at 15 minutes, 6 and 24 hours after the start of infusion.

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The mean clearance for the 6-hour data (with its 95% confidence interval) is estimated at 8.51 ml/min/kg (7.60 ml/kg/min, 9.42 ml/kg/min). The mean plasma clearance ( $\pm$  SD) at 6 and 24 hours of infusion are reported to be  $9.48 \pm 4.37$  ml/min/kg and  $19.91 \pm 32.66$  ml/kg/min. ***(Please note that the discrepancy between the values for clearance at the 6 hour time point of 8.51 ml/min/kg and 9.48 ml/min/kg is due to a difference in the number of subjects used to calculate the clearance values. The 8.51 ml/min/kg is obtained from data from 65 subjects whereas the value of 9.48 ml/min/kg was obtained from data 29 subjects. Please refer to tables 1 and 3.)*** The difference in the clearance values at the 2 time points approached significance ( $p=0.072$ ). Interestingly, a difference in the clearance values was also noted in study 704.306 (IV infusion study with 4 hour infusion time at doses of 0.025 and 0.05  $\mu$ g/kg/min). In that study, the difference in the clearance values approached significance,

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with the value for the clearance at the 0.05  $\mu\text{g}/\text{kg}/\text{min}$  being higher than that at half that dose.

Eight subjects had more than a doubling of their clearance values at 24 hours, as compared with the value of their clearance at 6 hours. These subjects are reported not to be unique with regard to their identifiable demographic factor, baseline clinical variable or concomitant medication use. In one of the subjects, the clearance value for Natreacor increased from 23.26 to 180.72 ml/kg/min from the 6 hour time point to the 24 hour time point and it is stated that this subject was a treatment failure and therefore switched to another therapeutic agent.

The sponsor reports that the analysis of the clearance at the 24-hour time point is rather problematic for several reasons, which will be listed as follows. First, the data from only 29 subjects, out of a possible 85 subjects taking Natreacor was included in the analysis, for reasons such as missing plasma values, drug discontinuation or dose modification due to side effects. The sponsor raises the possibility that these events may not have been random, thus creating a selection bias. Second, the endogenous levels of hBNP may be changing over time, due to an improvement in the clinical status of the subjects, whereas the value for the clearance was estimated by subtracting a constant value of endogenous hBNP from the plasma values at the 6- and 24-hour time points. Third, it is reported that the unnormalized clearance at the 6-hour time point is correlated with body weight, whereas the 24-hour unnormalized clearance is not correlated with body weight, leading the sponsor to conclude that the 24-hour data from the study do not provide a reliable basis for the definitive assessment of CL over time. Consequently, only the 6-hour values were used for further evaluation of the effect of demographic and clinical factors.

The following 3 tables show the data described above:

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