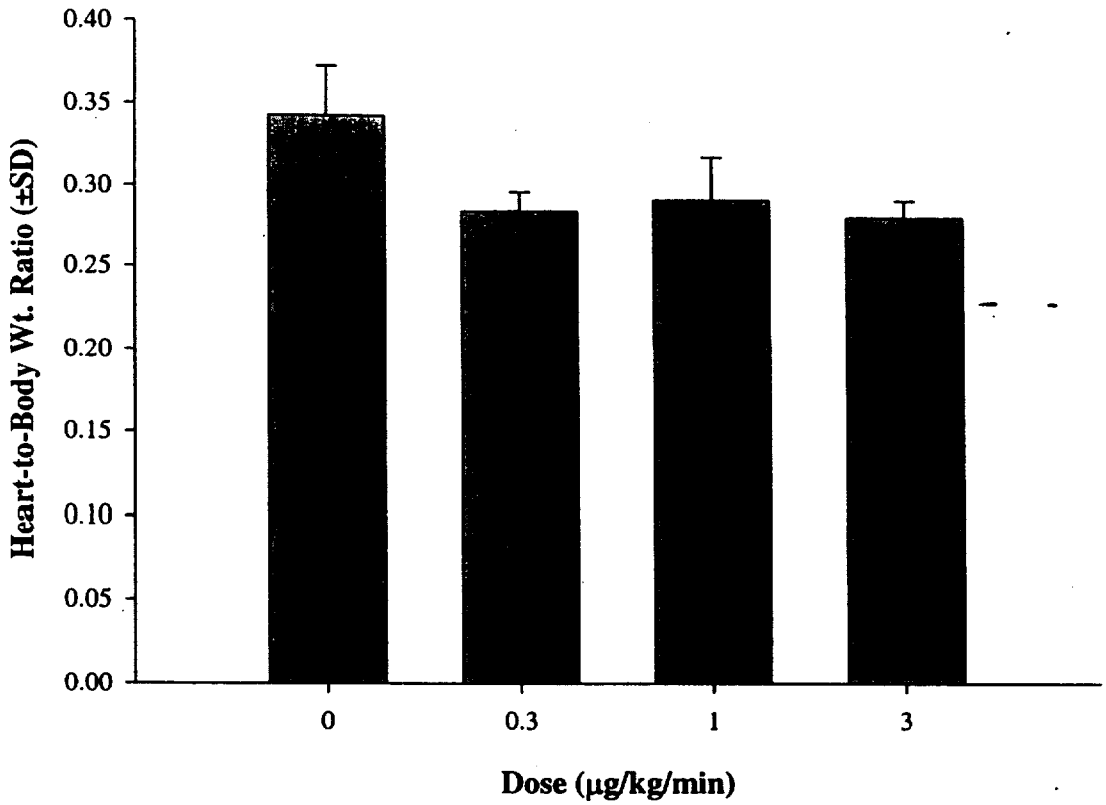
Figure 29

Effect of SC-70400 on Heart-to-Body Weight Ratios in Male Monkeys



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Drug steady state concentrations and plasma clearance values on Day 14 after two weeks of continuous infusion are shown in Table 7. Plasma BNP concentrations showed dose-proportionality and were relatively stable over the two week infusion period (Figure 30). The clearance values seen at the 0.3 μg/kg/min dose were significantly smaller then the values seen at the 1.0 and 3.0 μg/kg/min doses. This difference was thought to be due to inter-animal and/or assay variability.

Table 7

Steady State Concentrations (C_{ss})
and Plasma Clearance Values on Day 14
After Two-Week Infusion of hBNP in Monkeys

Dose (μg/kg/min)	Steady State Concentrations (ng/ml ± SD)	Clearance (ml/min/kg ± SD)
0.3		
1.0		
3.0		

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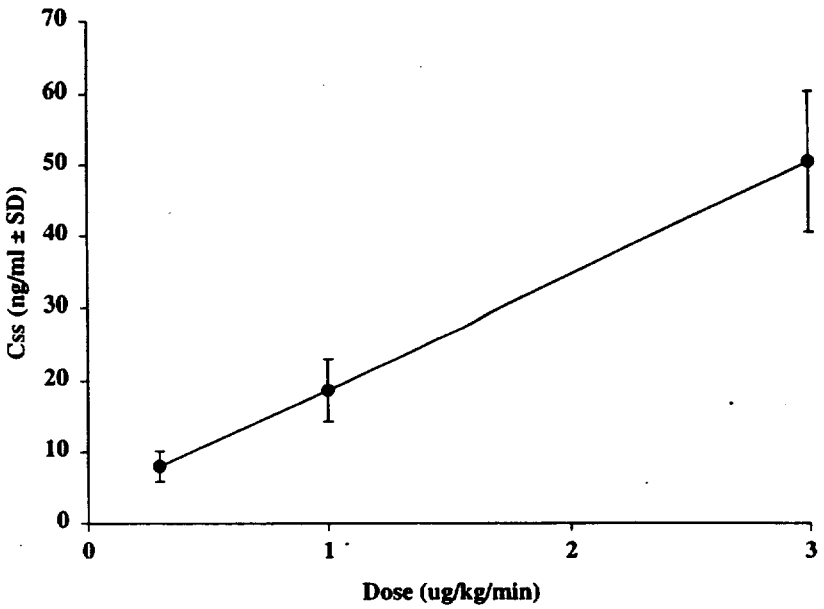
Figure 30

Steady-State Concentrations (Css) on Day 14
After Two-Week Infusion of hBNP in Monkeys (Mean ± SD)



Conclusions: Administration of SC-70400 to cynomolgus monkeys by continuous i.v. infusion for two weeks at dosages from 0.3 to 3.0 µg/kg/min resulted in minimal adverse effects. Body weights were minimally affected (5-6% weight loss vs 1-2% for controls). There were no drug-related effects during physical (respiration rates and rectal temperatures) or ophthalmic examination, and there were no drug-related effects on surface ECG measurements. Systolic, diastolic, and mean arterial blood pressures were reduced equally (approximately 20-25% when compared to untreated controls) at all doses beginning on Day 3 (first time point measured) in both males and females, an effect consistent with the relaxant or vasodilatory activity seen in precontracted cultures of human arterial and venous tissue. SC-70400 did not significantly alter sodium and chloride excretion and urine volume in spite of the drug's known natriuretic and diuretic effects. Except for the slightly reduced heart-to-body weight ratios in males, there were no other drug-related effects on organ weights. No microscopic findings related to drug-treatment were found. Plasma BNP concentrations showed dose-proportionality and were relatively stable over the two week infusion period. The high dose of 3.0 µg/kg/min represented a 200X multiple of the recommended human dose of 0.015 µg/kg/min. The steady state concentrations (postdose plasma hBNP concentrations minus predose hBNP plasma concentrations) achieved in monkeys at the high dose of 3.0 µg/kg/min (50.6 ng/ml) after 6 hours of continuous infusion were 24X the steady state concentrations (2.2 ng/ml) achieved in humans

at the recommended dose of 0.015 μg/kg/min after 6 hours of continuous infusion (Study No. 704.311).

3.2.3. Two-week continuous intravenous infusion toxicity study with recombinant hBNP and synthetic hBNP in Cynomolgus monkeys (Study No. 00185; Protocol No. 96-008-70400; Vol. 20 p. 104 to Vol. 21 p. 411):

Testing Facility: [redacted]
Protocol Number: 93-020-70400
Study Date(s): 6/17/96 to 7/15/96
GLP Compliance: Yes

Purpose: This study assessed and compared the toxicity of recombinant and synthetic hBNP when given by continuous infusion to cynomolgus monkeys for two weeks. Also assessed were reversibility of effects of recombinant hBNP after a 2-week recovery period, the hemolytic potential or recombinant hBNP for monkey and human whole blood, and compatibility of recombinant hBNP for monkey and human serum and plasma.

Methods: Male and female cynomolgus monkeys was administered either recombinant hBNP (rec-hBNP; lot no. H0001A1) or synthetic hBNP (syn-hBNP; lot no. G0004A1) in vehicle (5% dextrose) by intravenous infusion into the femoral vein (3 ml/kg/hr) for two weeks. Doses were adjusted based on the most recent body weight. Controls received vehicle. The study design is shown in the table below:

Study Design

			Number of Animals	
Group	**hBNP Dose (μg/kg/min)**	**Dose Conc. (μg/ml)**	**Male**	**Female**
Control (Vehicle)	0	0	6	6
Recombinant-Low	0.3	6	4	4
Recombinant-Mid	1.0	20	4	4
Recombinant-High	3.0	60	6	6
Synthetic	1.0	20	4	4

Two monkeys/sex/group from the Control and Recombinant High Dose groups, designated as recovery animals, were treated for two weeks, then drug treatment was discontinued, and the animals allowed to recover for two weeks to assess reversibility of any effects. Lactated Ringer's solution was infused during the two week recovery period.

Monkeys were observed twice daily for mortality and moribundity. Body weights were recorded before treatment, on the first day of treatment, and weekly thereafter. Physical examinations (including rectal body temperatures and respiration rates) were done on nonanesthetized animals before initiation of treatment (postsurgery) and once during Weeks 2 and 4. Electrocardiograms were done for each animal before initiation of treatment (postsurgery) and once during Weeks 1, 2, and 4. Leads I, II, III, aVR, aVL, and aVF were used. Blood

pressure measurements and heart rates were recorded on nonanesthetized animals before initiation of treatment (postsurgery) and once during Weeks 1, 2, and 4. Ophthalmic examinations were done on anesthetized animals before initiation of treatment (postsurgery) and once during Week 2. Blood and urine samples were collected from each animal twice before initiation of treatment, once during Weeks 1 and 2, and once during Week 4 (recovery) for standard hematology, clinical chemistry, and urinalysis.

Blood was collected for pharmacokinetic analysis from nonanesthetized animals before dosing on Day 1, at approximately 6 and 24 hours after beginning infusion with the test material or vehicle (Day 1), on Day 8, and on Day 15 before termination of infusion of test material or vehicle. Blood was also collected for analysis of antibodies to rec-hBNP and syn-hBNP from nonanesthetized animals before dosing on Day 1, on Day 15 before termination of infusion of test material or vehicle, and Day 29.

At the end of each study period (Day 15 for treatment animals and Day 30 for recovery animals), monkeys were sacrificed and a necropsy performed. The necropsy included a macroscopic examination of the external surface of the body, all orifices, the cranial cavity, the brain and spinal cord, the nasal cavity and paranasal sinuses, and the thoracic, abdominal, and pelvic cavities and viscera. Sixteen organs were weighed. Forty-five tissues were preserved in formalin, but only those tissues determined "as appropriate" were examined microscopically from the control and high dose rec-hBNP groups.

Hemolytic potential was determined by mixing vehicle or rec-hBNP at final concentrations up to 30 µg/ml in whole monkey or human heparinized blood, and incubating at 37° C for 45 min. The amount of hemoglobin in the supernatant was measured spectrophotometrically.

Blood compatibility was determined by mixing vehicle or rec-hBNP at final concentrations of 30 µg/ml in monkey or human serum or plasma, and incubating at 22.4° C for 30 min. Tubes were examined microscopically for precipitation or coagulation. If precipitation or coagulation were seen, the mixture was considered incompatible (positive test result).

Results: One female in the control group was pale, had sunken eyes, and was hypoactive. The animal was sacrificed on Day 4 after clinical pathology findings suggested an acute hemolytic anemia. No other deaths were reported. Although several of the animals were hypoactive during the drug treatment phase, it occurred with equal frequency across treatment groups. Clinical pathology findings indicated that a few of the animals were anemic by Week 2. The cause of the anemia was thought to be due to the frequent blood collections and reduced erythropoiesis secondary to inflammation associated with chronic catheterization.

Body weights in the drug treated females were higher than the female controls, and, therefore, were not considered to be toxicologically important.

There were no drug-related effects on rectal body temperatures, respiration rates, ECG measurements (no effects on conduction disturbances or heat rates and no arrhythmias), or ophthalmic observations.

The effect of hBNP on mean arterial blood pressures (MAP) is shown in Figures 31A (males) and 31B (females). As shown, there was no increase in effect with increasing dose. Blood pressures after two weeks of recovery remained below those of untreated controls. There were no significant differences on MAP between syn-hBNP and rec-hBNP when given at 1.0 µg/kg/min.

Figure 31A

Effect of rec-hBNP and syn-hBNP
on Mean Arterial Pressure (MAP) in Male Monkeys

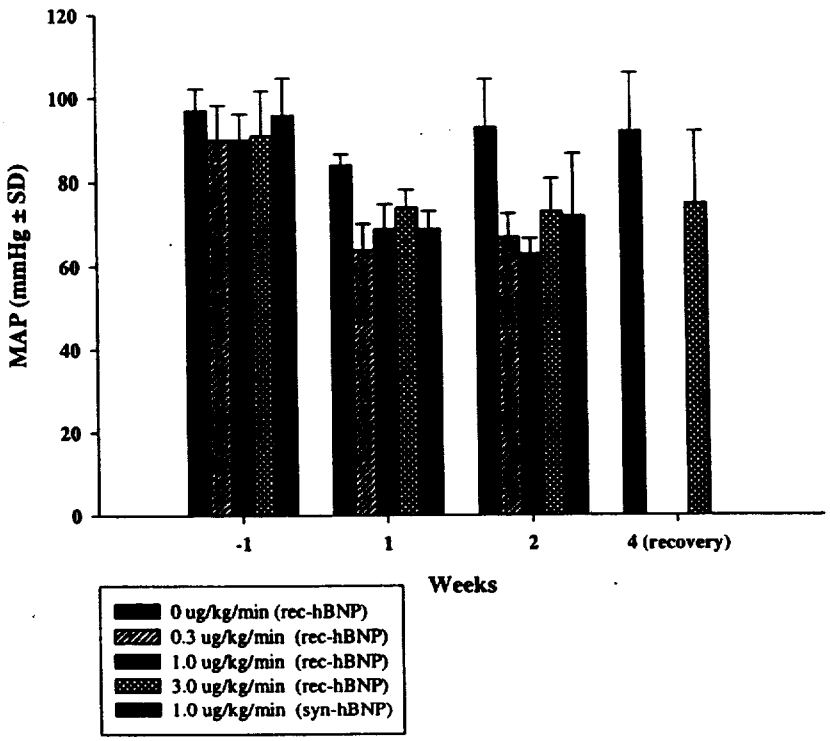


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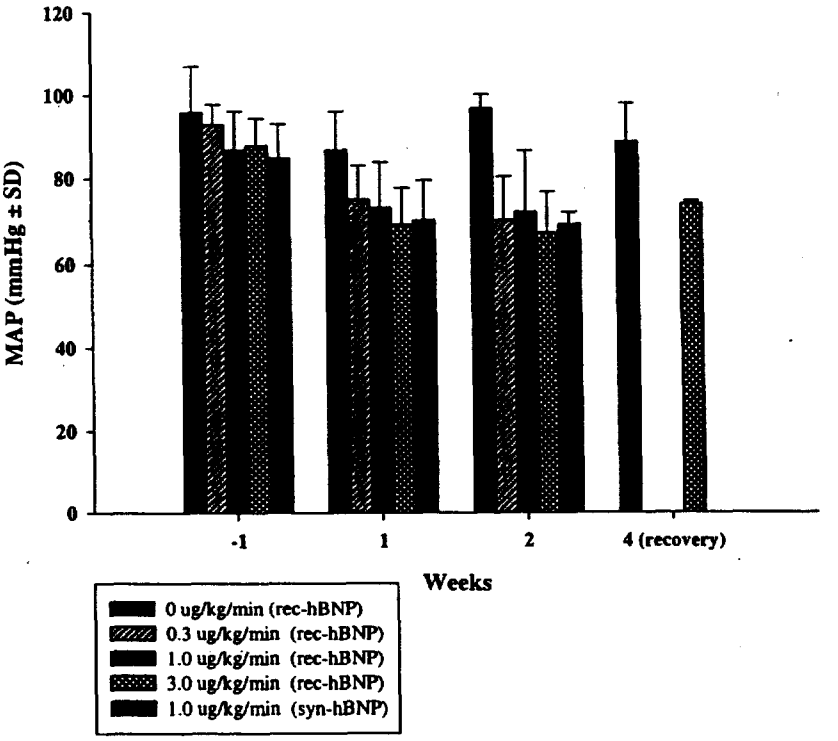
Figure 31B

Effect of rec-hBNP and syn-hBNP
on Mean Arterial Pressure (MAP) in Female Monkeys

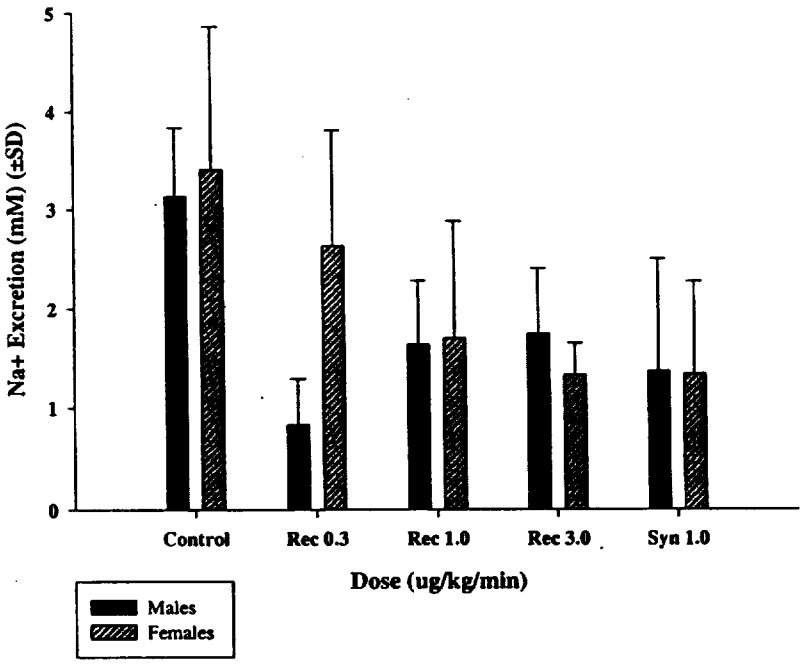


There were no readily apparent drug-related effects on clinical pathology parameters. Also, no differences were found between rec-hBNP-treated and syn-hBNP-treated animals. A few of the animals became moderately anemic by Week 2 with hematocrits of about 20%. The cause of the anemia was thought to be due to the frequent blood collections and to reduced erythropoiesis secondary to inflammation associated with chronic catheterization.

Of interest was the lower urine sodium excretion at Week 1 when compared to controls (Figure 32). These effects were less marked by Week 2 and absent by Week 4 (after 2 weeks of recovery). No explanation was offered to account for the effects at Week 1 given the known natriuretic activity of hBNP.

Figure 32

Effect of rec-hBNP and syn-hBNP
on Mean Urine Sodium Excretion in Monkeys
(Week 1)



There were some changes in organ-to-body weight ratios. These included lower heart weight ratios in drug-treated males and higher liver weight ratios in drug-treated females. Lower heart-to-body weight ratios were also seen in a previous two-week monkey toxicity study using syn-hBNP, and were attributed to decreased blood pressures. However, there were no macroscopic or microscopic changes in any of the organs to indicate drug-induced toxicity. The only microscopic changes reported were in the catheter sites (inflammation, fibrosis, and thrombus formation).

Rec-hBNP at a final concentration of 30 µg/ml did not cause hemolysis when mixed with whole monkey or human blood, and it did not cause precipitation or coagulation when mixed with monkey or human plasma or serum at the same final concentration.

Pharmacokinetic analyses showed that steady-state and dose-related hBNP plasma concentrations occurred within the first 6 hours of infusion. There was no accumulation of hBNP in plasma over time (6 hours to 15 days). There were essentially no differences in hBNP plasma concentrations between similar doses (1.0 µg/kg/min) of rec-hBNP and syn-hBNP. Steady-state plasma concentrations for rec-hBNP and syn-hBNP on Day 15 in male and female monkeys are shown in Table 8 and Figure 33.

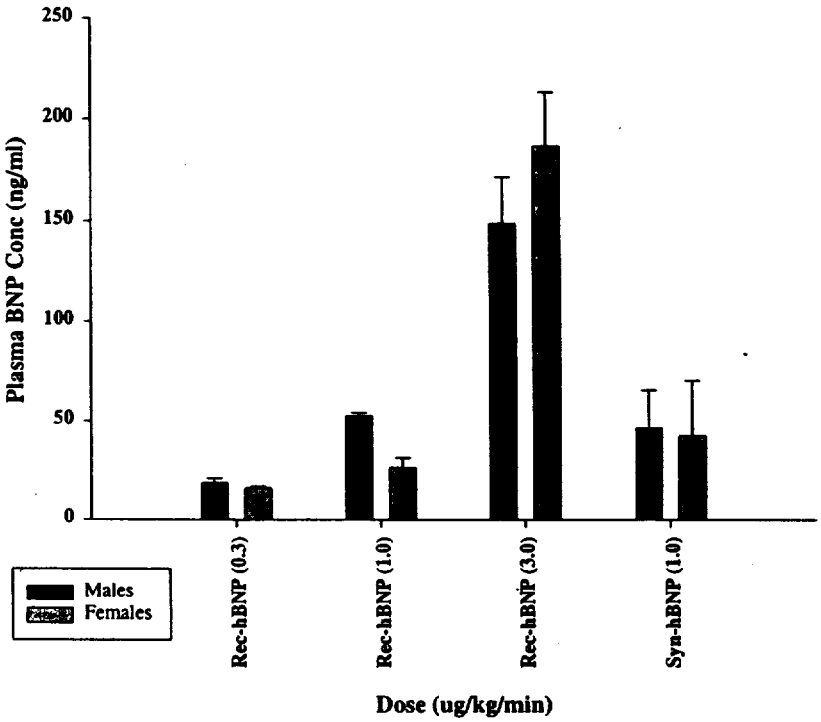
Table 8

Steady-State Concentrations (Css) on Day 15
After Two-Week Infusion of Rec-hBNP and Syn-hBNP in Monkeys (Mean ± SD)

Dose (μg/kg/min)	**Sex**	**Steady State Concentrations (ng/ml ± SD)**
0.3 (Rec-hBNP)	M	18.66 ± 2.46
	F	15.97 ± 0.99
1.0 (Rec-hBNP)	M	52.71 ± 11.55
	F	66.88 ± 5.06
3.0 (Rec-hBNP)	M	149.09 ± 23.27
	F	188.36 ± 25.39
1.0 (Syn-hBNP)	M	46.60 ± 19.04
	F	42.44 ± 28.19

Figure 33

Steady-State Concentrations (Css) on Day 15
After Two-Week Infusion of Rec-hBNP and Syn-hBNP in Monkeys (Mean ± SD



Conclusions: There were no remarkable clinical findings that could be attributed to drug treatment. Changes, such as hypoactivity, occurred with equal frequency across treatment groups. Some of the animals were anemic, but this was thought to be due to the frequent blood collections and reduced erythropoiesis secondary to inflammation associated with chronic catheterization.

hBNP reduced mean arterial blood pressures equally across all doses (0.3-3.0 µg/kg/min). Blood pressures after two weeks of recovery remained below those of untreated controls. There were no significant differences on MAP between syn-hBNP and rec-hBNP when given at 1.0 µg/kg/min.

Urine sodium excretion was lower in drug-treated animals by Week 1. No explanation was offered to account for the effects given the known natriuretic activity of hBNP, although decreased renal perfusion may be involved. The effect on lowering sodium excretion was absent after two weeks of recovery.

There were some changes in organ-to-body weight ratios. These included lower heart weight ratios in drug-treated males and higher liver weight ratios in drug-treated females. Lower

heart-to-body weight ratios were also seen in a previous two-week monkey toxicity study using syn-hBNP, and were attributed to decreased blood pressures. However, there were no macroscopic or microscopic changes in any of the organs to indicate drug-induced toxicity.

Rec-hBNP did not cause hemolysis when mixed with whole monkey or human blood, and it did not cause precipitation or coagulation when mixed with monkey or human plasma or serum.

Pharmacokinetic analyses showed that steady-state and dose-related hBNP plasma concentrations occurred within the first 6 hours of infusion. There was no accumulation of hBNP in plasma over time (6 hours to 15 days). There were essentially no differences in hBNP plasma concentrations between similar doses (1.0 μg/kg/min) of rec-hBNP and syn-hBNP.

3.3. Special Toxicity Studies:

3.3.1. Acute intravenous tolerance study with SC-70400 in rabbits (Study No. 00251; Protocol No. 93-022-70400; Vol. 22 pp. 2-120):

Testing Facility[ ]
Protocol Number: 93-022-70400
Study Date(s): 8/18/93 to 8/23/93
GLP Compliance: Yes

Purpose: This study assessed the local tolerance of the SC-70400 (hBNP) when administered by 1 hour infusions to rabbits as a single dose or as multiple doses.

Methods: Male Hra:(NZW)SPF rabbits (4/group; 2.4-2.7 kg) were given a daily one hour infusion of SC-70400 (lot no. E0013A) at either 20 μg/kg/hr (20 μg/ml) or 200 μg/kg/hr (200 μg/ml) in a marginal vein of the left ear for 5 consecutive days to assess the local tolerance of multiple doses. On Day 5, an additional one hour infusion was given at the same doses to the right ear to assess the effect after a single dose. Controls received vehicle (5% dextrose).

Rabbits and infusion sites were observed for signs of toxicity 15 and 30 min and 2 hours after each dose. On Day 6, rabbits were sacrificed and a partial necropsy was performed on the infusion sites. The infusion sites were then examined microscopically.

Results: There were no macroscopic findings. Microscopically, hemorrhage was found in two controls and three rabbits given 200 μg/kg/hr, and chronic inflammation in one control rabbit. These findings were attributed to mechanical trauma, and not to drug or vehicle treatment.

Conclusions: There was no local irritation in the infusion sites of rabbits given SC-70400 at doses up to 200 μg/kg infused over one hour each day for up to 5 consecutive days.

3.3.2. Determination of potential antibody formation to hBNP in rabbits (Study No. 00305; Protocol No. 97-006-704; Vol. 22 pp. 121-180):

Testing Facility[ ]
Protocol Number: 97-006-704
Study Date(s): Not given (>4/2/97)
GLP Compliance: Yes

Purpose: This study determined if anti-hBNP antibodies were generated in rabbits after repeated exposure to either recombinant hBNP (rec-hBNP) or synthetic hBNP (syn-hBNP).

Methods: Adult male Hra:(NZW)SPF rabbits (6/group; 2.2-2.4 kg) were given either 0.3 µg rec-hBNP/kg/min (lot no. H0003A1) or 0.3 µg rec-hBNP/kg/min (lot no. G0004A1) at a rate of 0.05 ml/kg/min over 8 hours by intravenous infusion using a syringe pump via a marginal ear vein on Days 1, 28, and 56 (= 4 week intervals). Blood was collected on Days -5 and on Days 8, 35, and 63 (= 7 days after each dose) for anti-hBNP antibody analysis. The anti-hBNP antibody assay used was an antigen displacement enzyme-linked immunosorbant assay (ELISA). The amount of anti-BNP antibodies bound was determined by comparing the amount of biotinylated BNP probe bound in the test serum sample to the amount of probe bound in a reference sample containing known amounts of recombinant hBNP monoclonal antibodies (standard curve).

Results: There was no measurable antibody response in any of the serum samples collected from rabbits prior to or following repeated administration with synthetic or recombinant hBNP. These results indicated that hBNP was not immunogenic in rabbits, and that the development of antibodies in humans, which can bind drug and reduce effectiveness, is not likely to occur.

3.3.3. Hemolytic potential and blood compatibility testing with SC-70400 (Study No. 00249; Protocol No. 93-021-70400; Vol. 22 pp. 181-214):

Testing Facility[ ]
Protocol Number: 93-021-70400
Study Date(s): 8/17/93
GLP Compliance: Yes

Purpose: This study examined the hemolytic potential of recombinant hBNP for monkey and human whole blood, and compatibility of recombinant hBNP for monkey and human serum and plasma. The study was conducted as part of a two week continuous intravenous infusion toxicity study (3.2.3. above) with recombinant hBNP and synthetic hBNP in Cynomolgus monkeys (Study No. 00185). [Note: In that study, blood hemoglobin was not elevated.]

Methods: Hemolytic potential was determined by mixing vehicle or rec-hBNP at final concentrations up to 30 µg/ml in whole monkey or human heparinized blood, and incubating at 37° C for 45 min. The amount of hemoglobin in the supernatant was measured spectrophotometrically.

Blood compatibility was determined by mixing vehicle or rec-hBNP at final concentrations of 30 µg/ml in monkey or human serum or plasma, and incubating at 22.4° C for 30 min. Tubes were examined microscopically for precipitation or coagulation. If precipitation or coagulation were seen, the mixture was considered incompatible (positive test result).

Results: Rec-hBNP at a final concentration of 30 µg/ml did not cause hemolysis when mixed with whole monkey or human blood, and it did not cause precipitation or coagulation when mixed with monkey or human plasma or serum at the same final concentration.

3.3.4. Mutagenicity test with rhBNP in the *Salmonella-Escherichia coli* / mammalian-microsome reverse mutation assay preincubation method (Study No. 00254; Protocol No. 96-015-704; Vol. 22 pp. 215-246):

Testing Facility[ ]
Protocol Number: 96-015-704
Study Date(s): 8/28/96 to 9/28/96
GLP Compliance: Yes

Purpose: This study examined the mutagenic potential of recombinant-hBNP using the Ames test. This assay evaluated the test article and/or its metabolites for their ability to induce reverse mutations at the histidine locus in the genome of specific *Samonella typhimurium* tester strains and at the tryptophan locus in the *Escherichia coli* strain WP2uvrA both in the presence and absence of an exogenous metabolic activation system of mammalian microsomal enzymes derived from AroclorTM-induced rat liver (S9).

Methods: The tester strains used in this study were *Salmonella typhimurium* tester strains TA98, TA1OO, TA1535, TA1537, and *Escherichia coli* tester strain WP2uvrA. The assay was conducted with five doses of recombinant-hBNP (lot no. H0001A1) in both the presence and absence of S9 mix along with concurrent vehicle and positive controls using three plates per dose. The doses tested in the mutagenicity assays were 112, 224, 448, 895, and 1790 µg of test article per ml of preincubation reaction mixture. Appropriate vehicle (deionized water) and positive controls for both with and without S9 were employed. All cultures were performed in triplicate.

Two methods of drug exposure were used. The first method used a preincubation exposure method in which bacteria were preincubated with drug ± S9 for 20 min at 37° C, mixed with molten agar, overlayed onto minimal agar, and incubated for 48 hours at 37° C at which time revertant colonies were counted. The second method used a treat and plate exposure method in which bacteria were exposed to drug ± S9 for 60 min at 37° C, centrifuged to remove drug and S9, resuspended in molten agar, overlayed onto minimal agar, and incubated for 48 hours at 37° C at which time revertant colonies were counted. A positive response for both drug and positive controls was defined as that which produced (1) a 2-3 fold increase (depending upon the tester strain used) in revertants over the number observed with vehicle, and (2) a dose response to increasing concentrations of drug.

Results: No increase in revertants were found with the *Escherichia coli* tester strain WP2uvrA using the preincubation method. With the *Salmonella typhimurium* tester strains

TA98, TA1535, and TA1537, an enhancement or overgrowth of the bacterial lawn was observed. This was thought to indicate that the drug (a recombinant peptide) was supplying additional histidine that would enhance the growth of bacteria in minimal-histidine media. The sponsor confirmed to the conducting laboratory that the test article did contain histidine (amounts not given). No increase in revertants were found with tester strains TA1535 or TA1537, but a small dose-related increase in the number of revertants was found at the highest concentration (1790 µg/ml) with tester strain TA98 both in the presence (1.9-fold) and absence (2.0-fold) of S9 (Figure 34). Positive controls showed the appropriate responses. Cultures of TA100 were not tested due to contamination.

To minimize the growth-enhancing effect of histidine in the test article preparation, a second assay method was used in which cultures of TA98 and TA100 were treated with drug ± S9, washed, and plated. This assay is a modification of the preincubation exposure method originally described by Green and Muriel (1976) in which the separation of the test article from the bacteria allows removal of test article components that could interfere with the selective conditions of the assay. Results using the treat and plate exposure method showed that no increase in revertants were found (Figure 35). TA100 was not retested using the preincubation exposure method.

Figure 34

Effect of Rec-hBNP on Mutagenicity in TA98
(Preincubation Exposure Method)

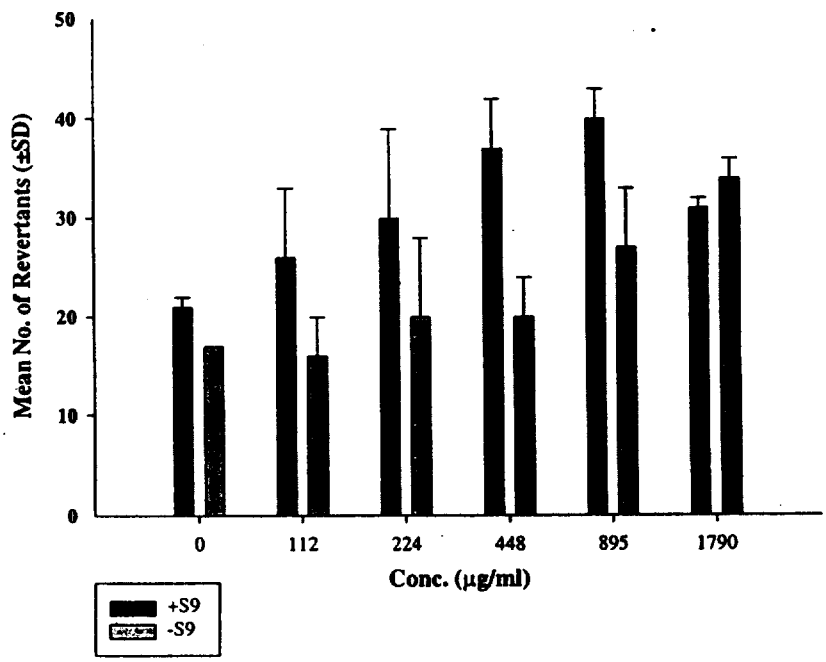
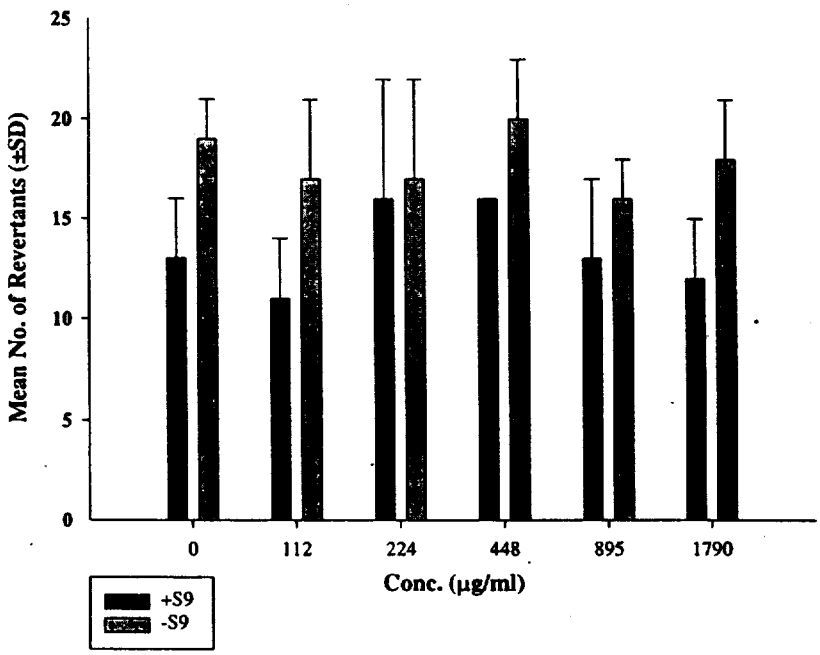


Figure 35

Effect of Rec-hBNP on Mutagenicity in TA98
(Treat and Plate Exposure Method)



Conclusions: Under the conditions defined for the study, recombinant human BNP did not cause a positive increase in the number of revertants in any of the bacterial tester strains either in the presence or absence of metabolic activation (±S9). The minimally positive results (1.9-2.0-fold increase in revertants) found with the *S. typhimurium* tester strain TA98 in the preincubation exposure method was not found when the method was modified to remove exposure to the test article during the growth phase of the assay (a published treat and plate exposure method; Green and Muriel, 1976).

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4. ABSORPTION; DISTRIBUTION, METABOLISM, AND ELIMINATION:

4.1. Pharmacokinetics and induction of plasma cyclic GMP following intravenous bolus and continuous infusion or recombinant and synthetic hBNP (Study No. 00181; Vol. 23 pp. 29-50):

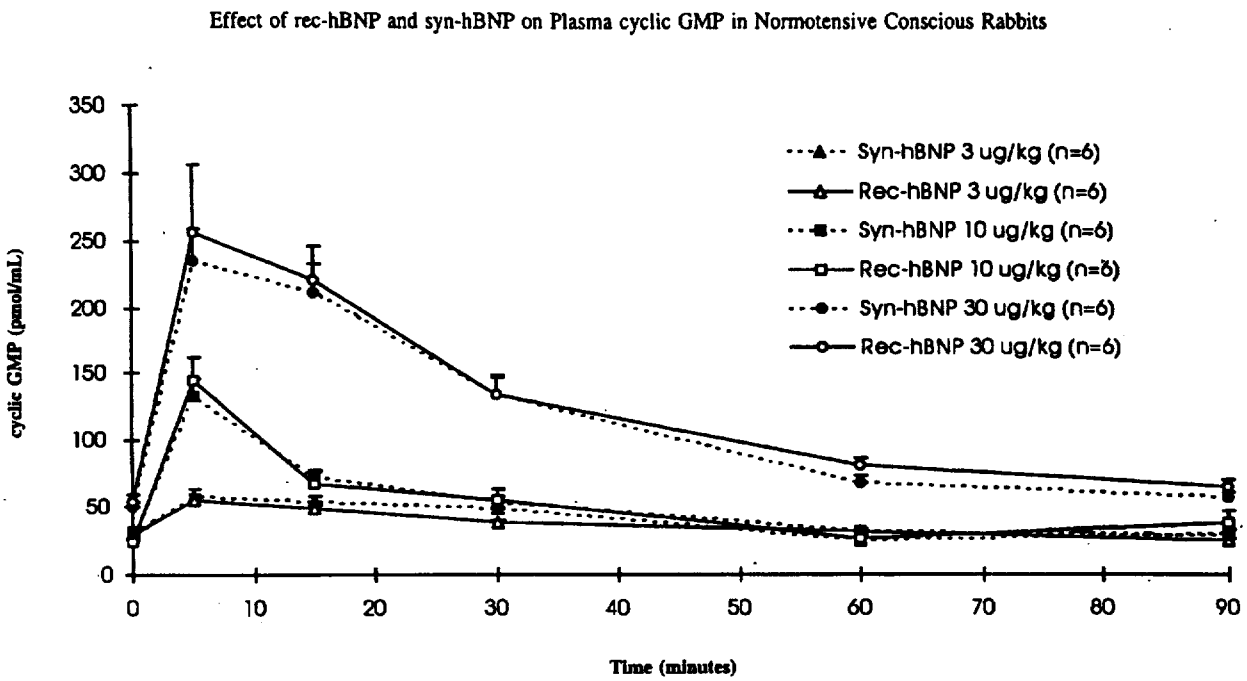
Purpose: hBNP binds and activates the guanylyl cyclase-A (GC-A) membrane receptor resulting in synthesis and intracellular accumulation of cyclic GMP (cGMP), much of which is released from the cell. Administration of hBNP to dogs and humans has been shown to result in increased plasma concentrations of cGMP. This study compared the ability of synthetic (syn) and recombinant (rec) hBNP to increase the plasma concentration of cyclic GMP after bolus and continuous infusion into rabbits. Pharmacokinetic data was also derived.

Methods: Conscious New Zealand White male rabbits (2.5-3.0 kg) were administered either syn-hBNP or rec-hBNP in two separate studies. In Study No. 1, rabbits (6/group) were given rec-hBNP or syn-hBNP at bolus doses of 3 μg/kg, 10 μg/kg, or 30 μg/kg. Blood was withdrawn at various times for up to 90 min for determination of cGMP and immunoreactive-hBNP levels. In Study No. 2, rabbits were given either syn-hBNP or rec-hBNP as an escalating dose, continuous infusion at 50, 100, and 200 ng/kg/min for 60 min at each dose. Blood was taken for determination of cGMP and immunoreactive-hBNP levels at various times after dosing (time 0, at 60 min for the 50 ng/kg/min dose, at 120 min for the 100 ng/kg/min dose, and at 180 min for the 200 ng/kg/min dose).

Plasma cGMP levels were determined by radioimmunoassay, and plasma hBNP levels were determined by an antigen displacement assay (ELISA).

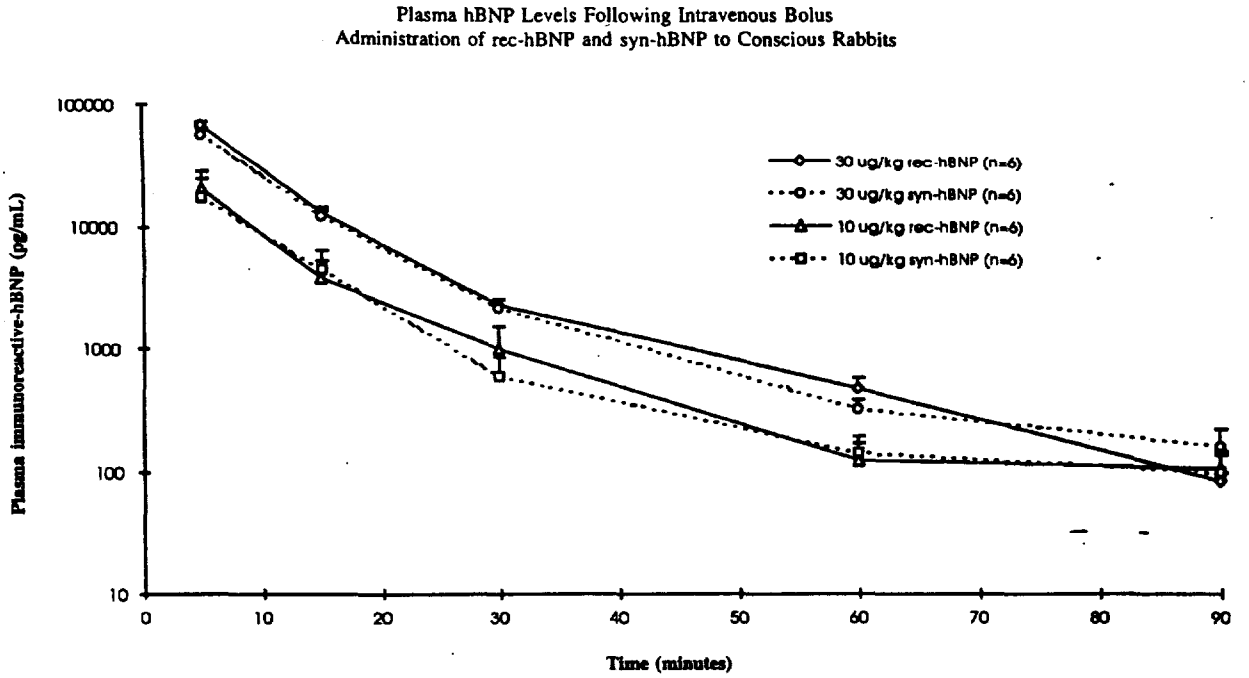
Results: Study No. 1 (i.v. bolus): Administration of rec-hBNP and syn-hBNP resulted in a time and dose-dependent increase in plasma cGMP concentrations (Figure 35; Sponsor's Figure 1). Plasma cGMP levels peaked within 15 min of administration and returned to baseline within 30-60 min. There was no significant difference in the levels of plasma cyclic GMP following syn-hBNP and rec-hBNP administration. An approximate half-life of 20.6 min for cGMP was derived.

Figure 35 (Sponsor's Figure 1)



Pharmacokinetic data showed that endogenous plasma hBNP levels were below the limit of detection (≤ 20 pg/mL) before drug administration. After administration of 10 µg/kg hBNP, plasma levels were best fit to a one compartment model that assumed drug concentrations declining exponentially in a first order process. After administration of 30 µg/kg hBNP, plasma hBNP levels were best fit to a two compartment model that predicted drug concentrations declining biexponentially as the sum of two first-order processes. The pharmacokinetic parameters derived from intravenous bolus administration of 10 µg/kg and 30 µg/kg of rec-hBNP and syn-hBNP were similar (Figure 36; Sponsor's Figure 3).

Figure 36 (Sponsor's Figure 3)



Study No. 2 (i.v. continuous infusion): Administration of rec-hBNP and syn-hBNP resulted in a dose-dependent increase in plasma cGMP concentrations (Figure 37). Levels of plasma cyclic GMP following administration of syn-hBNP and rec-hBNP were similar. Steady-state plasma concentrations of hBNP showed a dose-dependent increase. Metabolic clearance rates were similar for animals given rec-hBNP or syn-hBNP (Table 9; Sponsor's Table 8).

Figure 37

Effect of Continuous I.V. Infusion of Rec-hBNP and Syn-hBNP
on Plasma Cyclic GMP in Rabbits

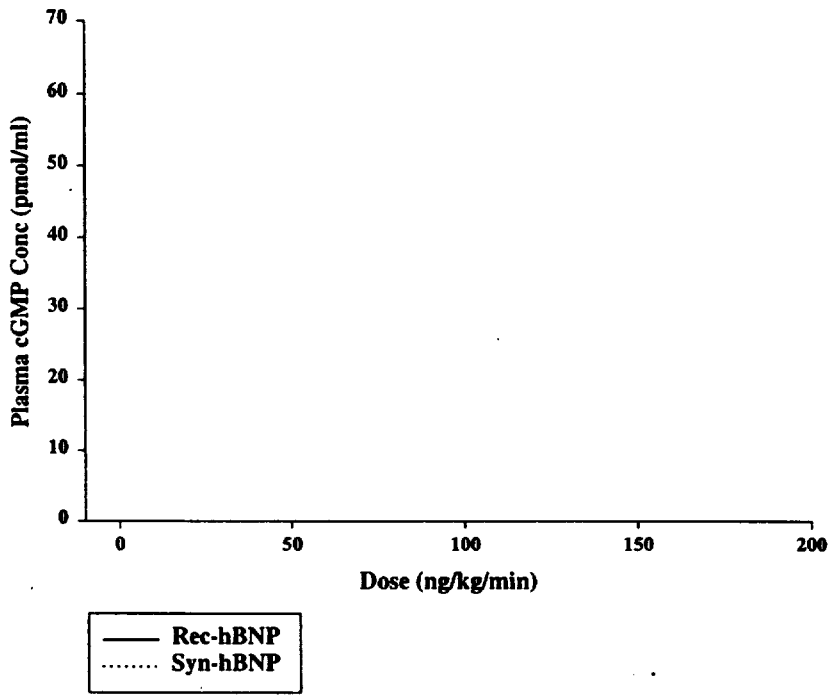


Table 9 (Sponsor's Table 8)

Table 8

Metabolic Clearance Rates (MCR) Derived from Intravenous Continuous Infusion of rec-hBNP and syn-hBNP in Conscious Rabbits

syn-hBNP		rec-hBNP	
Infusion Rate (ng/kg/min)	**MCR (mL/min-kg)**	**Infusion Rate (ng/kg/min)**	**MCR (mL/min-kg)**
38	53.8 ± 9.0	48.5	54.3 ± 0.7
76	47.9 ± 1.4	97	36.0 ± 3.6
152	33.0 ± 2.0	194	30.3 ± 2.1

Indicated doses of hBNP are net peptide.

Conclusions: Administration of rec-hBNP and syn-hBNP to rabbits by either bolus or continuous infusion resulted in increased plasma cGMP concentrations, an indication of its *in vivo* pharmacologic activity of guanylyl cylcase A receptor activation. Following bolus administration, plasma cGMP levels peaked within 15 min of administration and returned to baseline within 30-60 min with an approximate half-life of 20.6 min for cGMP. The pharmacokinetic parameters and levels of plasma cyclic GMP following bolus or continuous I.V. administration of syn-hBNP and rec-hBNP were similar.

4.2. Pharmacology and pharmacokinetics of recombinant and synthetic hBNP (Study No. 00184; Vol. 23 pp. 51-97):

Purpose: This study was a continuation of the previous study in which certain pharmacological properties of rec-hBNP and syn-hBNP were measured and compared in rabbits. In addition to the pharmacokinetics of hBNP and induction of cGMP following bolus and continuous I.V. infusion in conscious rabbits, this study also examined hBNP-induced activation of the human biological receptor (GC-A) in tissue culture, renal effects of bolus hBNP administration in anesthetized rabbits, and cardiovascular effects of bolus hBNP administration in anesthetized rabbits.

Methods: Tissue Culture: Chinese hamster ovary (CHO) cells expressing the human GC-A receptor were treated with either rec-hBNP or syn-hBNP at concentrations from 0.003 to 300 nM for 1.5 hours at 37° C. Control cultures were treated with vehicle (phosphate buffered saline with isobutylmethylxanthine and 2% bovine serum albumin). At the end of incubation, the culture supernatants were assayed for cyclic GMP concentrations using an enzyme-linked immunoassay.

Anesthetized rabbits: Male New Zealand White rabbits (9/group; 2.5-3.0 kg) were anesthetized and catheterized to the renal artery for collection and analysis of cardiovascular responses (heart rates and systolic, diastolic, and mean arterial pressures) over 10 min periods. Urine was collected in 20 min intervals for urinary parameters (volume, sodium, and potassium). Rabbits were treated with either rec-hBNP or syn-hBNP in increasing i.v. bolus doses of 1, 3, and 10 μg/kg.

Conscious rabbits: New Zealand White male rabbits (6/group; 2.5-3.0 kg) were administered either syn-hBNP or rec-hBNP in two separate studies. In Study No. 1, rabbits (6/group) were given rec-hBNP or syn-hBNP at bolus doses of 3 μg/kg, 10 μg/kg, or 30 μg/kg. Blood was withdrawn at various times for up to 90 min for determination of cGMP and immunoreactive-hBNP levels. In Study No. 2, rabbits were given either syn-hBNP or rec-hBNP as an escalating dose, continuous infusion at 50, 100, and 200 ng/kg/min for 60 min at each dose. Blood was taken for determination of cGMP and immunoreactive-hBNP levels at various times after dosing (time 0, at 60 min for the 50 ng/kg/min dose, at 120 min for the 100 ng/kg/min dose, and at 180 min for the 200 ng/kg/min dose).

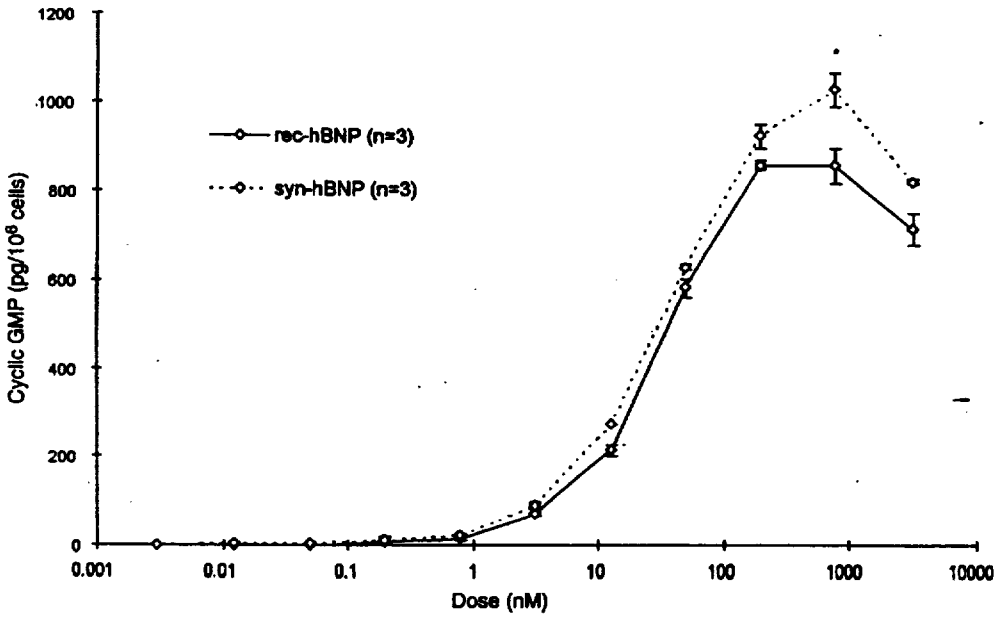
Plasma cGMP levels were determined by radioimmunoassay, and plasma hBNP levels were determined by an antigen displacement assay (ELISA).

Results: Tissue culture: Levels of cGMP released from CHO cells expressing the human GC-A receptor after treatment with either syn-hBNP or rec-hBNP were similar, except at the higher concentrations (≥ 800 nM) where syn-hBNP tended to produce greater cGMP release than rec-hBNP at equimolar concentrations (Figure 38; Sponsor's Figure 1).

Figure 38 (Sponsor's Figure 1)

Figure 1

Activation of the Human GC-A Receptor in Tissue Culture by recombinant hBNP (rec-hBNP) and synthetic hBNP (sec-hBNP)



* P < 0.05 rec-hBNP vs. syn-hBNP using unpaired two-tailed t-test.

Anesthetized rabbits: Intravenous bolus administration of increasing doses of rec-hBNP or syn-hBNP resulted in transient and dose-dependent increases in urine volume (Figure 39; Sponsor's Figure 6), urine sodium excretion rate (Figure 40; Sponsor's Figure 7), and urine potassium excretion rate. The renal effects occurred within the first 20 min collection period, and returned to baseline within another 20 min. There were no significant differences in renal effects between syn-hBNP and rec-hBNP.

Figure 39 (Sponsor's Figure 6)

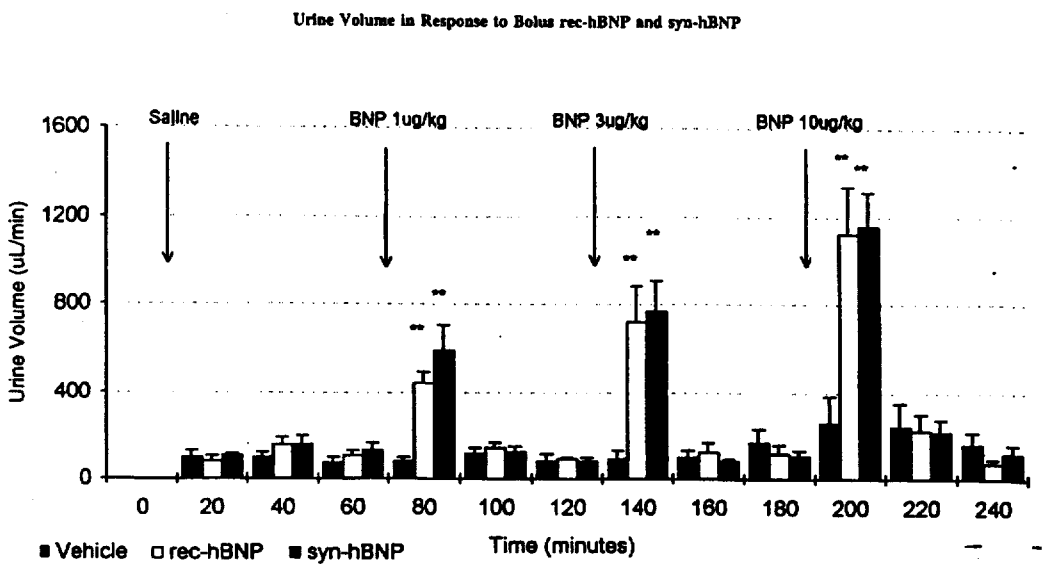
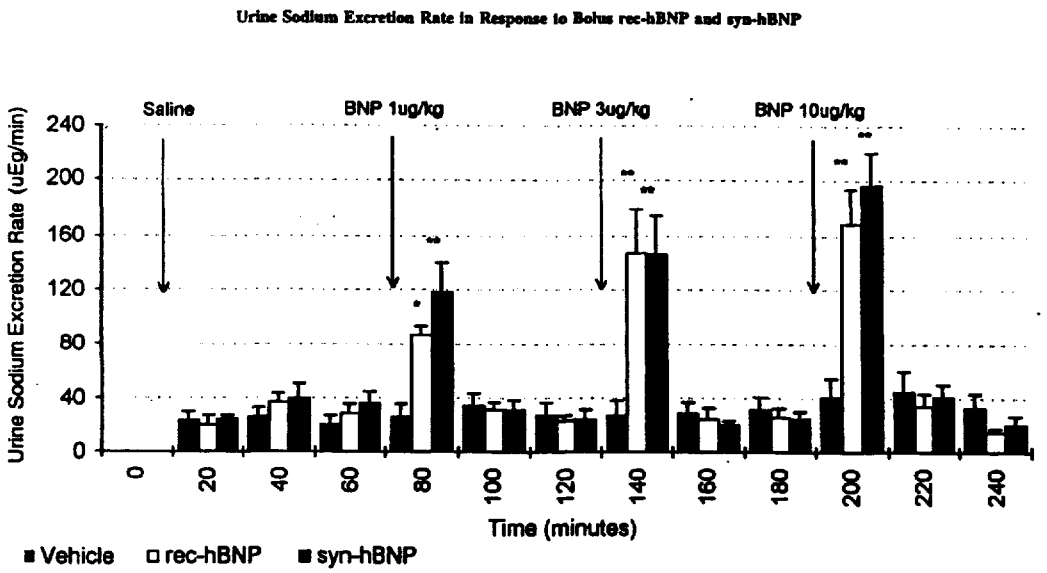


Figure 40 (Sponsor's Figure 7)



Intravenous bolus administration of increasing doses of rec-hBNP or syn-hBNP resulted in transient decreases in systolic, diastolic, and mean arterial blood pressures. Figure 41 (Sponsor's Figure 11) shows the mean arterial blood pressure data. Heart rates increased in all groups, saline and drug, over the course of the experiment (Figure 42; Sponsor's Figure 13). Heart rates in drug-treated rabbits were slightly higher than the controls, but this was not statistically significant. There were no significant differences in cardiovascular effects (blood pressure or heart rate) between syn-hBNP and rec-hBNP.

Figure 41 (Sponsor's Figure 11)

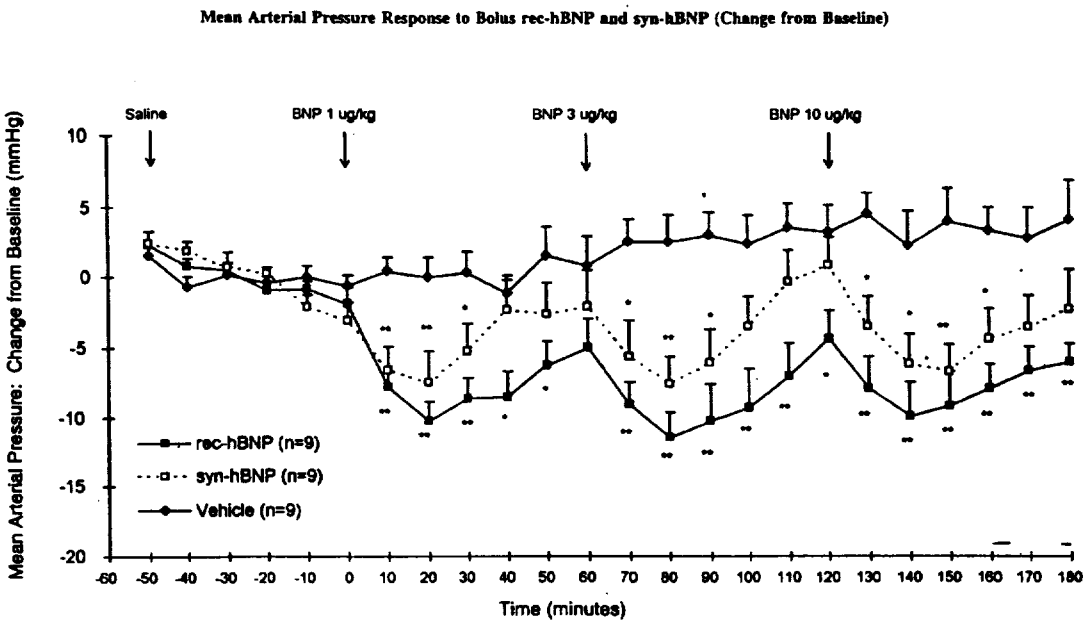
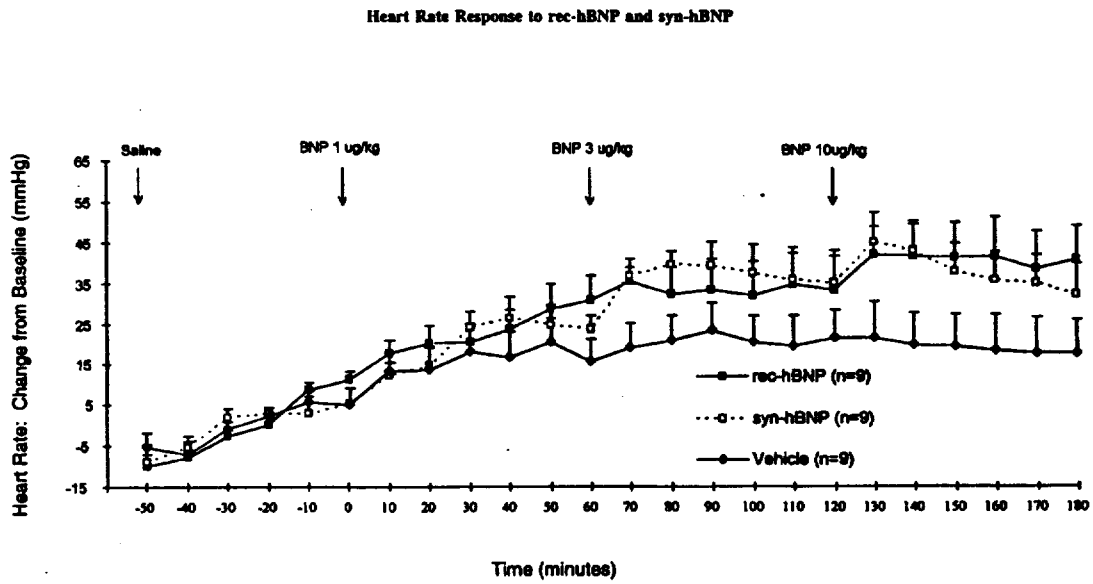


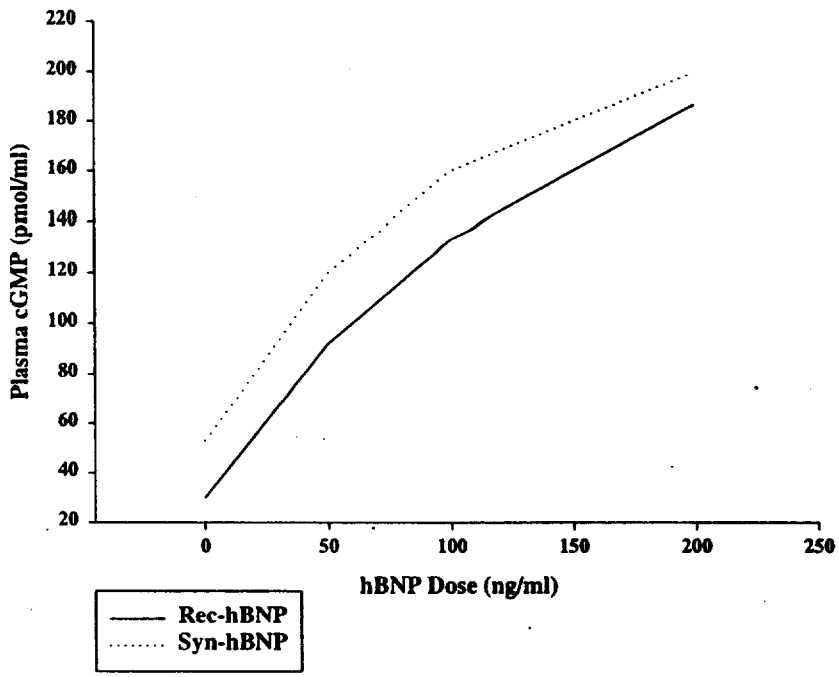
Figure 42 (Sponsor's Figure 13)



Conscious rabbits: Continuous infusion of rec-hBNP and syn-hBNP resulted in a dose-dependent increase in plasma cyclic GMP (Figure 43). Differences between syn-hBNP and rec-hBNP were minimal. Similar results were found with bolus administration (data not shown).

Figure 43

Induction of Plasma Cyclic GMP by Continuous Infusion
of Rec-hBNP and Syn-hBNP in Conscious Rabbits



Pharmacokinetic data are summarized in Table 10 (Sponsor's Table 14). Plasma hBNP values derived from animals treated with 10 and 30 μg/kg of rec-hBNP and syn-hBNP were fit to a two compartment model which assumed that drug concentrations declined exponentially as the sum of two first-order processes. The half-life values for the α phase ($t1/2\alpha$, 3.7 ± 1.0 minutes and 3.3 ± 0.4 minutes) and β phase ($t1/2\beta$, 15 ± 0.5 minutes and 15.5 ± 2.7 minutes) derived for rec-hBNP and syn-hBNP, respectively, were equivalent. There were no statistically significant differences in the other derived pharmacokinetic values including: micro rate constants (k_{12} ,k_{21}, and k_{13}), volumes of distribution (V_c ,V_{dss} , and V_{darea}), clearance, and the area under the plasma hBNP concentration-time curve.

Table 10 (Sponsor's Table 14)

Pharmacokinetics of rec-hBNP and syn-hBNP

	$t_{1/2\alpha}$ (min)	$t_{1/2\beta}$ (min)	k_{12} (min-1)	k_{21} (min-1)	k_{13} (min-1)	V_c (L/kg)	V_{dss} (L/kg)	CL (L/min-kg)	V_{darea} (L/kg)	$AUC_{(0-90)}$ (ng-min/mL)
30 µg/kg hBNP										
rec-hBNP										
Mean	4.2	15.4	0.013	0.050	0.209	0.142	0.092	0.015	0.351	1262
Std Dev	1.4	2.9	0.004	0.010	0.022	0.123	0.022	0.004	0.154	396
syn-hBNP										
Mean	3.5	16.2	0.012	0.046	0.215	0.129	0.131	0.022	0.508	858
Std Dev	1.2	1.9	0.001	0.005	0.015	0.067	0.030	0.004	0.064	146
10 µg/kg hBNP										
rec-hBNP										
Mean	3.7	15.0	0.019	0.053	0.186	0.087	0.117	0.016	0.344	480
Std Dev	1.0	0.5	0.007	0.002	0.017	0.025	0.032	0.004	0.089	101
syn-hBNP										
Mean	3.3	15.5	0.015	0.051	0.189	0.107	0.140	0.020	0.447	348
Std Dev	0.4	2.7	0.002	0.009	0.027	0.019	0.022	0.002	0.089	46

Plasma hBNP values were fitted to a two compartment model assuming drug concentrations decline biexponentially as the sum of two first-order processes as described by the formula:

$$Ct = A \exp(-\alpha \cdot t) + B \exp(-\beta \cdot t)$$

Values for $t_{1/2\alpha}$ and $t_{1/2\beta}$ were calculated from $0.693/\alpha$ and 0.693β, respectively. Micro rate constants, k_{12}, k_{21} and k_{13}, were calculated for each animal using the formulas:

$$k_{12} = A \cdot B \cdot (\beta-\alpha)2 / [(A+B) \cdot (A \cdot \beta + B \cdot \alpha)] \qquad k_{21} = (A \cdot \beta + B \cdot \alpha) / A+B \qquad k_{13} = A + B / (A/\alpha + B/\beta)$$

Pharmacokinetic parameters, clearance (CL), volume of distribution of the central compartment (V_c), volume of distribution at steady-state (V_{dss}), and volume of distribution by area (V_{darea}) were derived using the formulas:

$$V_c = \text{Dose} / A+B \qquad V_{dss} = [k_{12} + k_{21}) / K_{21}] \cdot V_c \qquad V_{darea} = (k_{13} \cdot V_c) / \beta \qquad CL = k_{13} \cdot V_c$$

Steady-state plasma hBNP concentrations derived for both recombinant and synthetic hBNP dosing groups were equivalent for each of the three doses tested (Table 11; Sponsor's Table 17).

Table 11 (Sponsor's Table 17)

Steady-state Plasma hBNP Concentration Following Continuous IV Infusion of rec-hBNP and syn-hBNP

hBNP Dose (ng/kg/min)	rec-hBNP Plasma hBNP (pg/mL)	syn-hBNP Plasma hBNP (pg/mL)
50		
100		
200		

Conclusions: Several studies were performed *in vitro* and *in vivo* to examine the equivalence of rec-hBNP and syn-hBNP.

Levels of cGMP released from CHO cells expressing the human GC-A receptor after treatment with either syn-hBNP or rec-hBNP were similar.

In anesthetized rabbits, intravenous bolus administration of increasing doses of rec-hBNP or syn-hBNP resulted in transient and dose-dependent increases in urine volume, urine sodium excretion rate, and urine potassium excretion rate, and in transient decreases in systolic, diastolic, and mean arterial blood pressures. There were no significant differences in renal or cardiovascular effects between syn-hBNP and rec-hBNP.

In conscious rabbits, continuous or bolus infusion rec-hBNP and syn-hBNP resulted in dose-dependent increases in plasma cyclic GMP. Plasma hBNP values were fit to a two compartment model which assumed that drug concentrations declined exponentially as the sum of two first-order processes. The half-life values for the α phase and β phase derived for rec-hBNP and syn-hBNP, respectively, were equivalent, as were half-life values, micro-rate constants, volumes of distribution, and clearance. Steady-state plasma hBNP concentrations derived for both recombinant and synthetic hBNP dosing groups were equivalent for each of the three doses tested.

4.3. Cardiovascular and renal actions and pharmacokinetics of Natrecor hBNP administered intravenously and subcutaneously to rabbits (Study No. 00123; Vol. 23 pp. 98-120):

Purpose: This study examined the cardiovascular (blood pressures and heart rates) and renal (urine volume and sodium excretion) effects of recombinant hBNP administered to anesthetized rabbits by bolus subcutaneous and intravenous injection. Pharmacokinetic parameters were also determined.

Methods: Male New Zealand White rabbits (9-12/group; 1.9-2.5 kg) were anesthetized and catheterized to record heart rate and systolic and diastolic blood pressures.

After stabilization for 30-60 min, rabbits were injected with rec-hBNP at 30 µg/kg by either the intravenous or subcutaneous routes. Controls received saline (vehicle). Blood samples were taken for determination of hBNP levels using an enzyme-linked immunoassay, and urine was collected for determination of urine volume, urine sodium, and urine potassium.

Results: After intravenous administration, systolic and diastolic blood pressures were decreased, while heart rates were increased (Figure 44; Sponsor's Figure 1). The decrease in blood pressure reached a nadir 40 min after administration. Similar results were obtained following subcutaneous administration, although the decrease in blood pressure was more pronounced and prolonged (Figure 45; Sponsor's Figure 2).

Figure 44 (Sponsor's Figure 1)

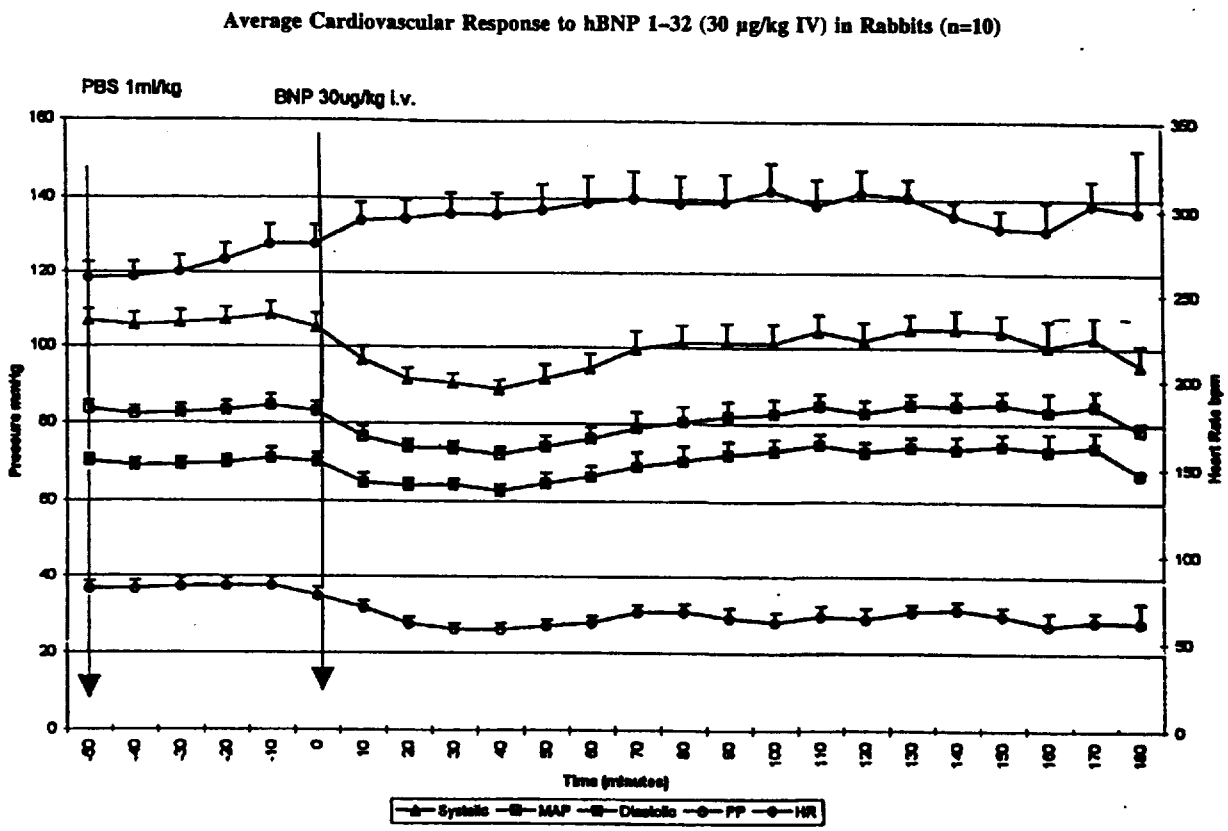


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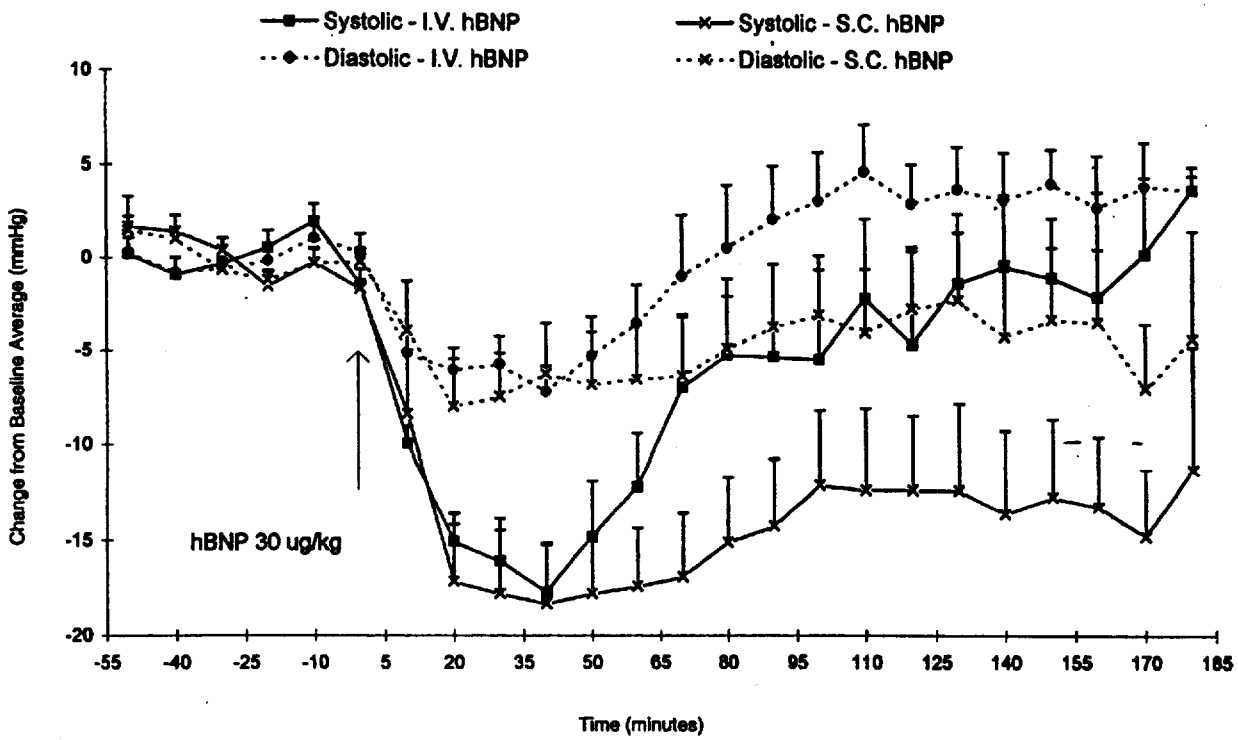
Figure 45 (Sponsor's Figure 2)

Figure 2

Average Change in Systolic and Diastolic Pressure Following IV or SC Administration of hBNP 1–32 in Rabbits



After intravenous administration, urine volume and urine sodium were increased with most of the increase occurring during the first two 20 min collection periods (Figure 46; Sponsor's Figure 4). Similar results were obtained following subcutaneous administration, although the increase in urine volume and urine sodium were more prolonged (data not shown). Effects on urine potassium excretion were not as marked following either intravenous or subcutaneous administration.

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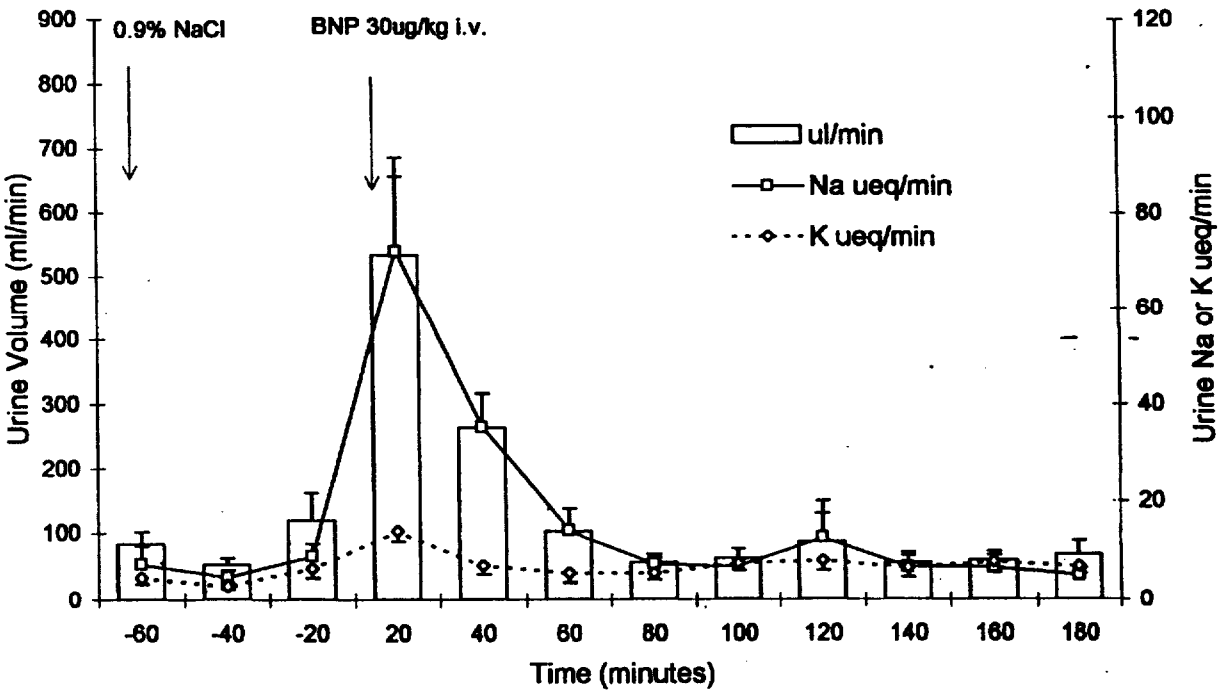
Figure 46 (Sponsor's Figure 4)

Figure 4

Average Renal Response to hBNP 1–32 (30 µg/kg IV) in Rabbits (n=9)



Pharmacokinetic data are summarized in Table 12 (Sponsor's Table 1). Subcutaneous administration resulted in about 65% of the drug (AUC) delivered as compared to intravenous administration. The t1/2α was 5.5 min and the t1/2β was 27.4 min. These PK data from rabbit were compared to dog and human (Table 13; Sponsor's Table 2).

Table 12 (Sponsor's Table 1)

Summary of Pharmacokinetics of hBNP in Rabbits Following Bolus IV or SC Doses

Route of Delivery	Cp_{max} (ng/mL)	t_{max} (min)	AUC (min • ng/mL)
Intravenous	92 ± 15*	2*	995*
Subcutaneous	8 ± 2	15–30	656

All values given are with standard deviation.

* These values assume that the 2-minute blood sample, the first sample taken, represented the highest circulating concentration.

Table 13 (Sponsor's Table 2)

Comparison of hBNP Pharmacokinetics in Rabbit, Canine, and Human

Pharmacokinetic Parameter	Rabbit	Canine	Human*
$t_{1/2}$ α (min)	5.5 ± 0.9	6.9 ± 1.6	
$t_{1/2}$ β (min)	27.4 ± 9.7	33.2 ± 6.7	
Vc (L/kg)	0.22 ± 0.05	0.24 ± 0.02	
Vd_{ss} (L/kg)	0.28 ± 0.06	0.473 ± 0.05	
CL (L/hr/kg)	1.54 ± 0.20	1.38 ± 0.07	

* 20 µg/kg/dose

Conclusions: Both intravenous and subcutaneous administration of recombinant hBNP to rabbits resulted in decreased systolic and diastolic blood pressures, increased heart rates, and increased urine volume and urine sodium excretion. Effects on urine potassium excretion were not as marked. hBNP delivered by the subcutaneous route was biologically active. The vascular and renal effects were more prolonged following subcutaneous administration, due to slower clearance. The loss of hBNP from the plasma compartment was fit to a two-compartment (α and β) model in rabbit, dog, and human with similar half lives.

4.4. Assessment of angiotensin converting enzyme on Natrecor hBNP metabolism *in vivo* and *in vitro* (Study No. 00112; Vol. 23 pp. 121-165):

[Note: This study was reviewed above (see Section 2.15, page 41).]

4.5. Pharmacokinetics of brain natriuretic peptide following a two-hour intravenous infusion of hBNP in the Cynomolgus monkey (Study No. 00245; Protocol No. 93-008-70400; Vol. 23 pp. 166-204):

Purpose: This study evaluated the pharmacokinetics of hBNP during a two-hour i.v. infusion to cynomolgus monkeys.

Methods: Three male cynomolgus monkeys were given hBNP (lot no. BN004) by i.v. infusion at 0.1, 0.3, or 1.0 g/kg/min for two hours at 0.5 ml/kg/hour according to the following schedule:

Monkey No.	Treatment I (μg/kg/min) (Day 1)	Treatment II (μg/kg/min) (Day 15)	Treatment III (μg/kg/min) (Day 29)
1011	0.1	0.3	1.0
2011	0.3	1.0	0.1
3011	1.0	0.1	0.3

Blood was collected before dosing and at various times up to 120 min after the start of infusion. Each monkey received saline i.v. at a volume equivalent to the volume of blood withdrawn. A radioimmunoassay kit was used to quantitate BNP in the plasma samples. Noncompartmental and compartmental analyses were conducted.

Results: There were technical problems due to low recovery of blood from monkey #3011 given 0.3 μg/kg/min. Pharmacokinetic parameters calculated from noncompartmental and compartmental analyses are shown in Tables 15 and 16, respectively (Sponsor's Tables 1 and 2, respectively). Mean plasma concentration-time curves of BNP at each dose are shown in Figure 47 (Sponsor's Figure 1). Results showed that BNP was cleared from the plasma relatively rapidly with a $t1/2\alpha$ of 2.7 ± 0.6 min and a $t1/2\beta$ of 26.3 ± 13.4 min. Clearance (CL) values decreased with increasing dose while volume of distribution at steady-state (Vss) values were similar at all doses.

Table 15 (Sponsor's Table 1)

Non-Compartmental Analysis

Pharmacokinetic Parameters of BNP Following a Two-Hour Infusion of hBNP to Monkeys

	C_{ss} (pg/mL)	β (min^{-1})	$t_{1/2,\beta}$ (min)	AUC (ng-min/mL)	CL (mL/min/kg)	V_{ss} (L/kg)	MRT (min)
0.1 µg/kg/min dose group							
1011	1940	0.023	29.8	222.7	56.6	0.45	8.0
2011	2882	0.046	15.0	338.6	30.7	0.43	13.9
3011	2409	0.031	22.2	247.4	44.1	0.09	2.1
Mean	2410	0.033	22.3	269.6	43.8	0.32	8.0
S.D.	471	0.012	7.4	61.0	13.0	0.20	5.9
0.3 µg/kg/min dose group							
1011	11752	0.036	19.0	1349.6	20.2	0.30	14.8
2011	10735	0.028	25.1	1203.3	32.2	0.41	12.9
Mean	11244	0.032	22.1	1276.5	26.2	0.36	13.9
S.D.	719	0.006	4.3	103.4	8.5	0.08	1.3
1 µg/kg/min dose group							
1011	54815	0.034	20.7	6187.1	18.4	0.21	11.7
2011	42905	0.036	19.4	4902.2	24.3	0.43	17.5
3011	46873	0.033	21.3	5372.1	26.1	0.35	13.4
Mean	47988	0.034	20.5	5487.1	22.9	0.33	14.2
S.D.	5722	0.002	1.0	650.1	4.0	0.11	3.0

Table 16 (Sponsor's Table 2)

Compartmental Analysis

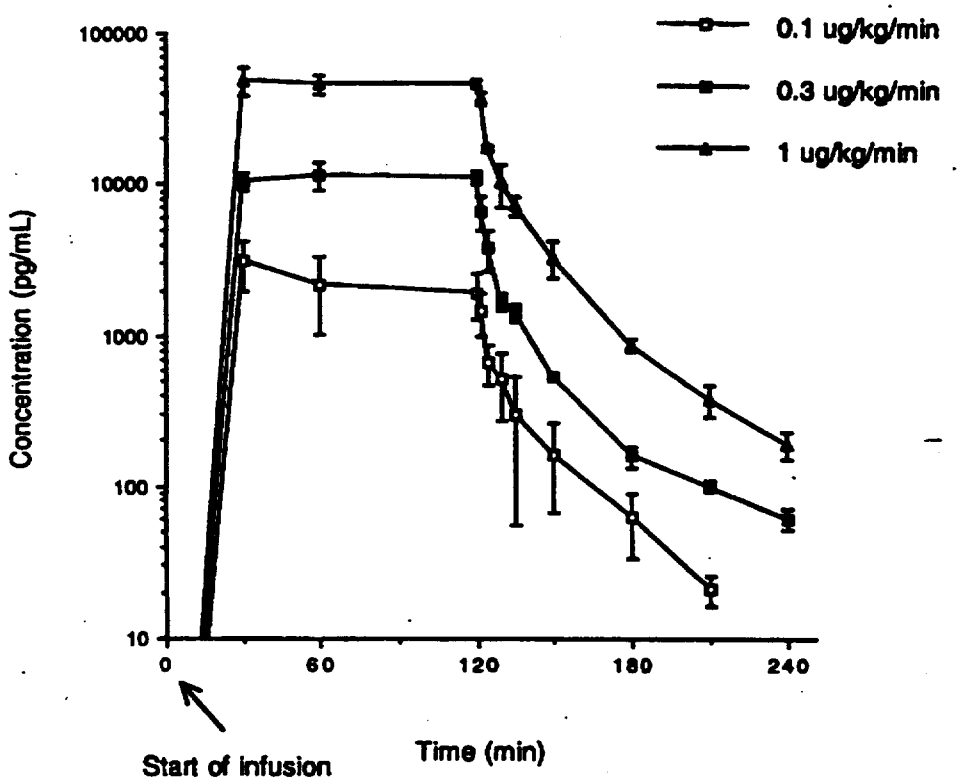
Pharmacokinetic Parameters of BNP Following a Two-Hour Infusion of hBNP to Monkeys

	R* (pg/mL)	S* (pg/mL)	α* (min^{-1})	β* (min^{-1})	$t_{1/2,\alpha}$ (min)	$t_{1/2,\beta}$ (min)
0.1 µg/kg/min dose group						
1011	1806	188	0.230	0.022	3.0	31.0
2011	1930	1090	0.431	0.046	1.6	15.2
3011	2534	55	0.248	0.012	2.8	57.4
Mean	2090	444	0.303	0.027	2.5	34.5
S.D.	389	563	0.111	0.017	0.8	21.3
0.3 µg/kg/min dose group						
1011	9863	1604	0.256	0.031	2.7	22.6
2011	9769	1414	0.285	0.029	2.4	24.0
Mean	9816	1509	0.271	0.030	2.6	23.3
S.D.	66	134	0.021	0.001	0.2	1.0
1 µg/kg/min dose group						
1011	44692	11922	0.254	0.040	2.7	17.5
2011	37621	7023	0.180	0.033	3.8	20.8
3011	41369	7651	0.263	0.031	2.6	22.0
Mean	41227	8865	0.232	0.035	3.0	20.1
S.D.	3538	2666	0.046	0.005	0.7	2.3

* R, S are the coefficients obtained from stripping the post infusion data, α; β are first-order macro-elimination rate constants for the two compartments.

Figure 47 (Sponsor's Figure 1)

Mean (±SD) Plasma Concentration-Time Curve of BNP
During and Following a Two-Hour Infusion of hBNP to Monkeys



4.6. The effect of the kidney, the natriuretic peptide clearance receptor, and peptidase activity on the plasma elimination of hBNP in rabbits (Study No. 00276; Vol. 23 pp. 205-236):

Purpose: Natriuretic peptide clearance (NP-C) receptors and neutral endopeptidase (NEP) are apparently abundantly expressed in the kidney, and the kidney may be involved in BNP clearance by metabolism or by filtration. The NP-C receptor mediates the cellular internalization and subsequent lysosomal degradation of ANP and possibly BNP, and BNP has been shown to be a substrate for NEP. This study used restricted blood flow to the kidney, blockade of the natriuretic peptide clearance (NP-C) receptor with a specific agonist, and inhibition of neutral endopeptidase (NEP) to examine the role of the kidney, the NP-C receptor, and NEP digestion, respectively, on the elimination of hBNP from plasma in rabbits. Also, *in vitro* studies examined whether hBNP was a substrate for human NEP.

Methods: Kidney: Male New Zealand White rabbits (6/group; 2.5-3.0 kg) were anesthetized then underwent surgery to completely restrict renal blood flow by ligation of both renal arteries. Control rabbits were subjected to sham surgery. In the first study, recombinant hBNP (lot no. 4132-51) was given as a constant infusion at 100 ng/kg/min which was initiated 60 min before renal artery ligation and continued for another 60 min after surgery was completed. Blood was collected before infusion and up to 120 min after drug infusion to

measure steady-state plasma levels of hBNP before and after renal artery ligation. In the second study, renal artery ligated and sham control rabbits were given an i.v. bolus dose of hBNP at 10 μg/kg. Blood was collected before and up to 120 min after drug infusion to measure post-surgical plasma levels of hBNP.

Natriuretic peptide clearance (NP-C) receptor: Conscious rabbits (6/group) were given an infusion of hBNP at 20 ng/kg/min for 4 hours. One hour after the start of drug infusion, C-ANP (a truncated form of ANP that binds to the NP-C receptor but not to the guanylyl cyclase-A receptor) was infused at escalating doses of 0.1, 1.0, and 10.0 μg/kg/min for one hour at each dose. Blood was taken before and up to 270 min after the start of hBNP infusion.

Neutral endopeptidase (NEP): Conscious rabbits (6/group) were given an infusion of hBNP at 20 ng/kg/min for 4 hours. One hour after the start of drug infusion, phosphoramidon, a neutral endopeptidase inhibitor, was infused at 25 μg/kg/min for two hours, at which time the dose was increased to 50 μg/kg/min for 2 hours. During the last hour of phosphoramidon and hBNP infusion, C-ANP was infused at 10 μg/kg/min. Blood was collected before and up to 240 min after start of hBNP infusion. A separate set of control rabbits were infused with only hBNP and C-ANP at the last hour of the 4-hour infusion (phosphoramidon was discontinued for the last hour).

For the *in vitro* studies, recombinant hBNP or hANP were mixed with recombinant human neutral endopeptidase (NEP) in buffer and incubated at 37° C. Aliquots were taken at up to 120 min after incubation, and analyzed for intact hBNP or hANP by [redacted]

Results: Kidney: The mean steady-state plasma hBNP values increased 1.9-fold from 5232 ± 2030 before renal artery ligation to 9653 ± 3945 after renal artery ligation ($P < 0.07$), whereas in sham operated controls there was no significant difference in plasma hBNP values before and after surgery (Figure 48). After a bolus injection of hBNP, plasma hBNP values were significantly higher ($P < 0.05$) in ligated rabbits than in sham-operated controls (Figure 49; Sponsor's Figure 1). Half-life values ($t_{1/2}\alpha$ and $t_{1/2}\beta$) were not significantly different between the two groups. These results indicated that the kidney played a role in the clearance of hBNP in rabbits.

Figure 48

Mean Steady-State Plasma hBNP Values in Rabbits
Before and After Complete Restriction in Renal Blood Flow

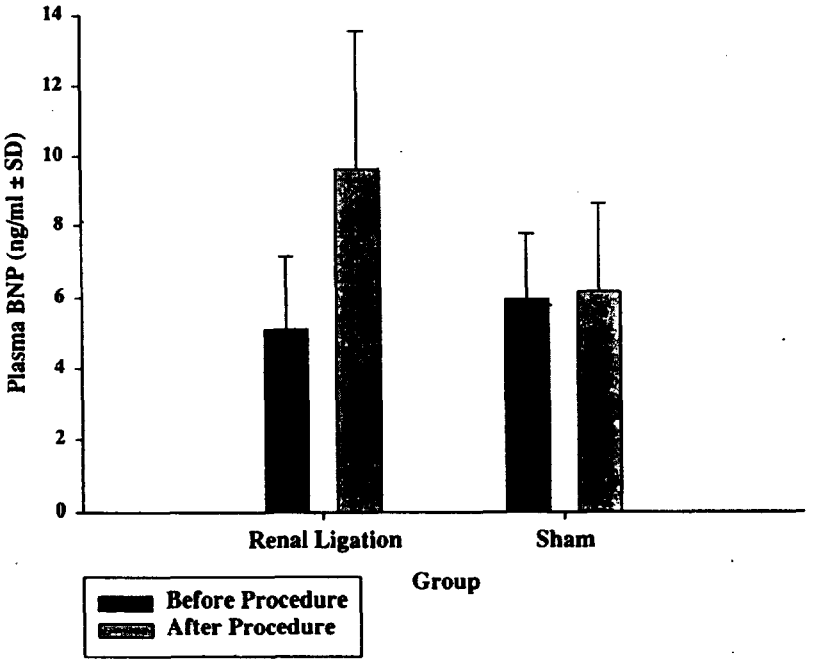
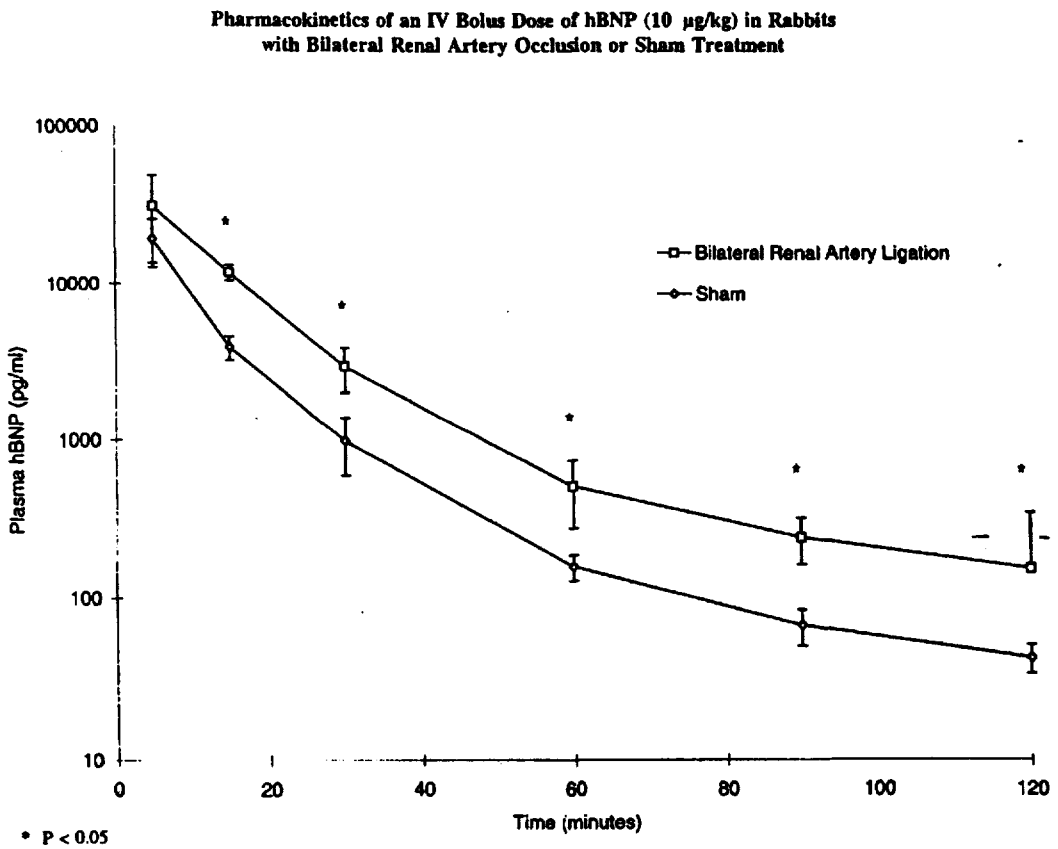
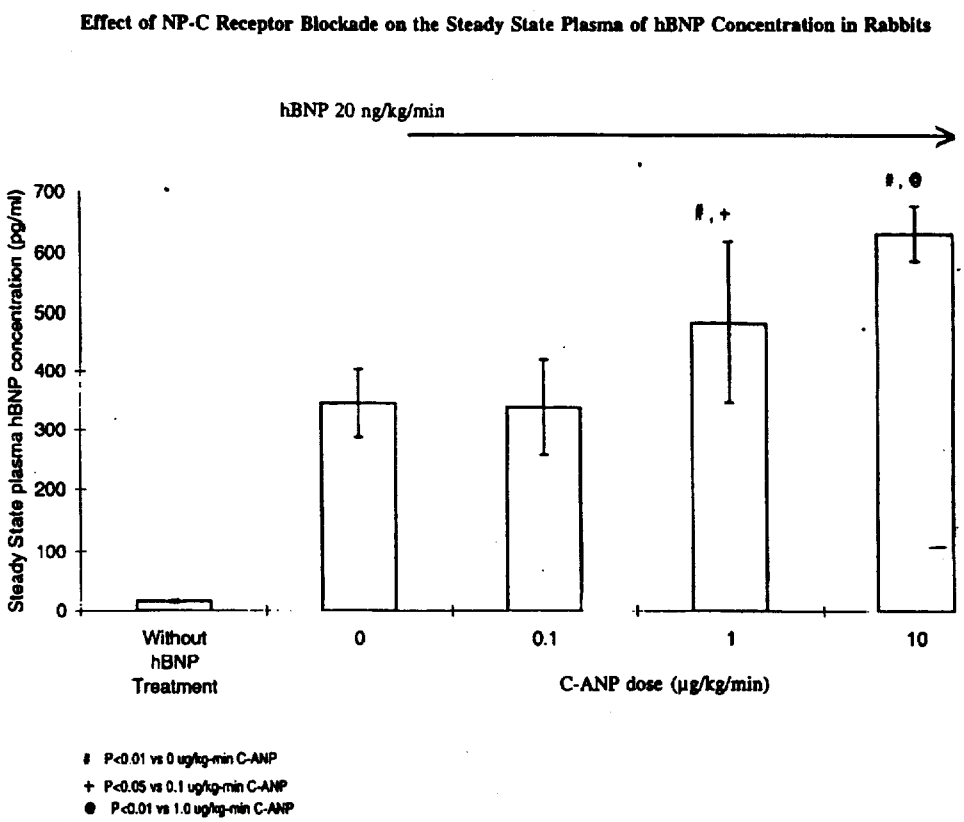


Figure 49 (Sponsor's Figure 1)



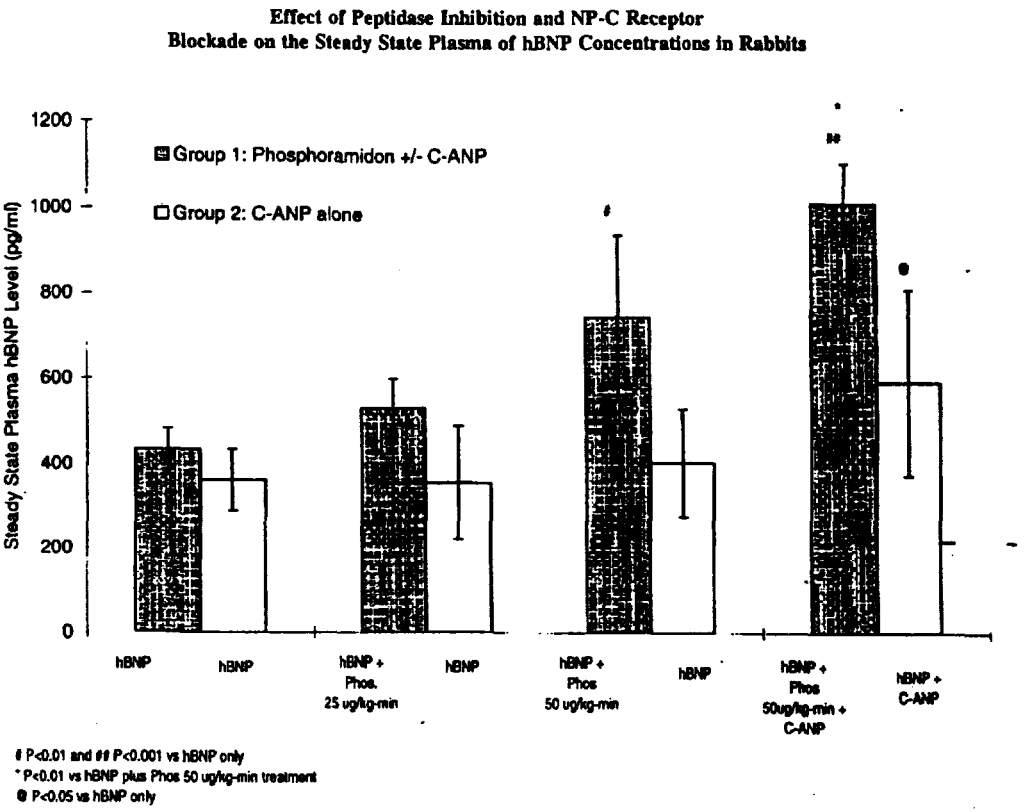
Natriuretic peptide clearance (NP-C) receptor: Mean steady-state plasma hBNP concentrations in rabbits increased 1.4 and 1.9-fold following co-infusion of the NP-C receptor agonist, C-ANP, at doses of 1.0 and 10.0 µg/kg/min, respectively (Figure 50; Sponsor's Figure 2). These results indicated that the NP-C receptor was involved in the elimination of plasma hBNP in rabbits.

Figure 50 (Sponsor's Figure 2)



Neutral endopeptidase (NEP): Mean steady-state plasma hBNP concentrations in rabbits increased 1.7-fold following co-infusion of the NEP-specific inhibitor phosphoramidon (Figure 51; Sponsor's Figure 3). When rabbits were treated with both phosphoramidon and C-ANP, the mean steady-state plasma hBNP concentrations further increased to 2.4-fold. Treatment with C-ANP alone increased mean steady-state plasma hBNP concentrations the same as with phosphoramidon alone (1.7-fold). These results *in vivo* indicated that neutral endopeptidases were involved in the elimination of hBNP in rabbits and that this effect was synergistic with the blockade of the NP-C receptor with C-ANP, suggesting that both pathways were involved in elimination of hBNP in rabbits.

Figure 51 (Sponsor's Figure 3)



Results from the *in vitro* studies showed that incubation of hBNP with recombinant NEP resulted in a reduction of the amount of intact hBNP in the mixture. Also, the rate of digestion of hANP was greater than that for hBNP. This suggested that hBNP was more resistant to peptidase digestion than hANP, and this was thought to help explain the differences between the clearance (hBNP = 9.8 ml/min/kg and ANP = 63.3 ml/min/kg) and volume of distribution (hBNP = 0.2 L/kg and ANP = 1.76 L/kg) for hBNP and Auriculin in human studies.

Conclusions: Studies were conducted to show that several pathways were involved in the elimination of hBNP from plasma in rabbits. Increases in mean steady-state plasma hBNP concentrations (reduced plasma clearance) resulted from: (1) reduced renal filtration or proteolysis after ligation of the renal arteries to restrict renal blood flow; (2) reduced receptor binding, internalization, and subsequent lysosomal degradation after co-infusion of the natriuretic peptide clearance (NP-C) receptor agonist C-ANP; and (3) reduced proteolysis after co-infusion of the neutral endopeptidase (NEP)-specific inhibitor phosphoramidon. *In vitro* studies showed that hBNP was more resistant to peptidase digestion than hANP.

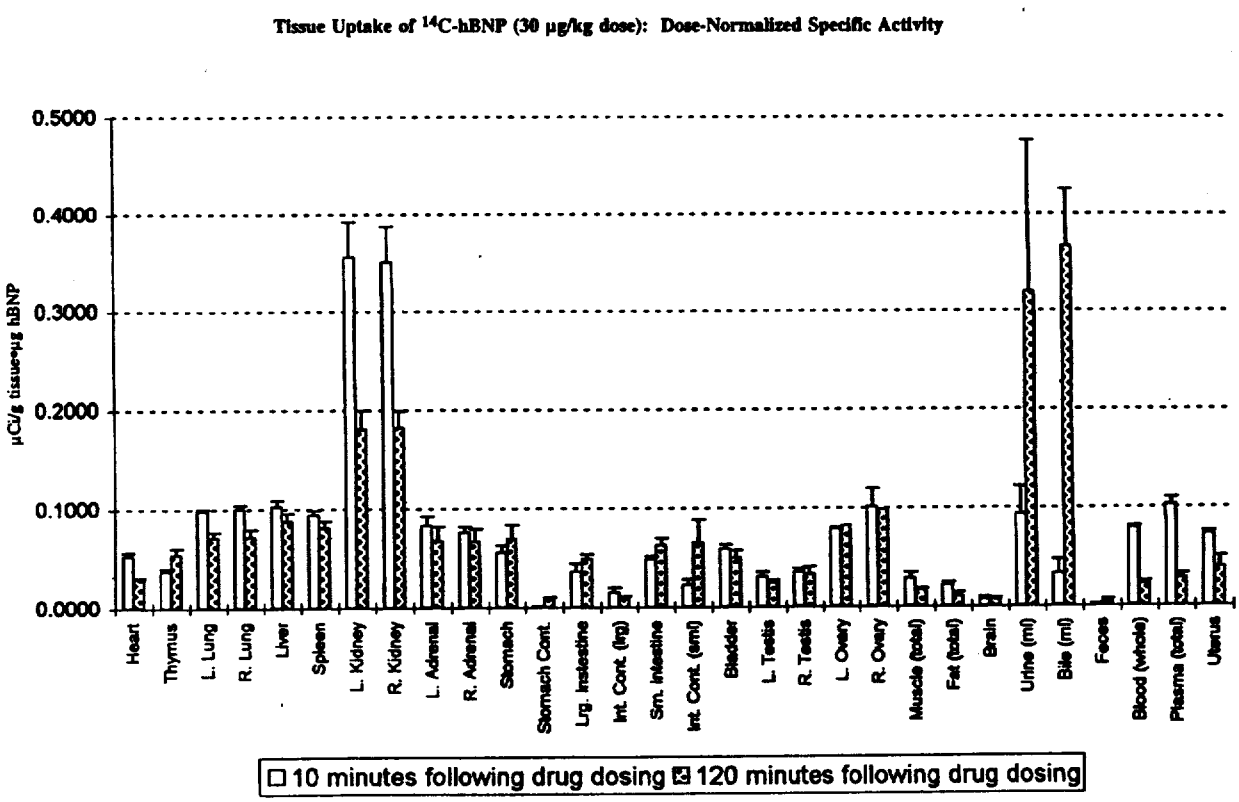
4.7. Tissue distribution of [^{14}C]-hBNP following bolus intravenous administration in rabbits (Study No. 00273; Vol. 23 pp. 237-266):

Purpose: This study measured the tissue distribution of radiolabeled synthetic hBNP when administered to rabbits as a bolus intravenous injection. The rabbit was chosen because its pharmacokinetics and hemodynamic effects of hBNP were similar to those in humans.

Methods: Male and female New Zealand White rabbits (3/sex/group) were injected into the ear vein with synthetic ^{14}C-hBNP at doses of either 10 µg/kg (4 males and 3 females/group) or 30 µg/kg (3/sex/group). After 10 or 120 min, rabbits were euthanized, organs removed and weighed, and samples of tissues were processed for radioactivity measurements. Data were expressed as either radioactivity per gm of tissue per µg hBNP administered (dose-normalized specific activity) or as a percent of the total dose per organ.

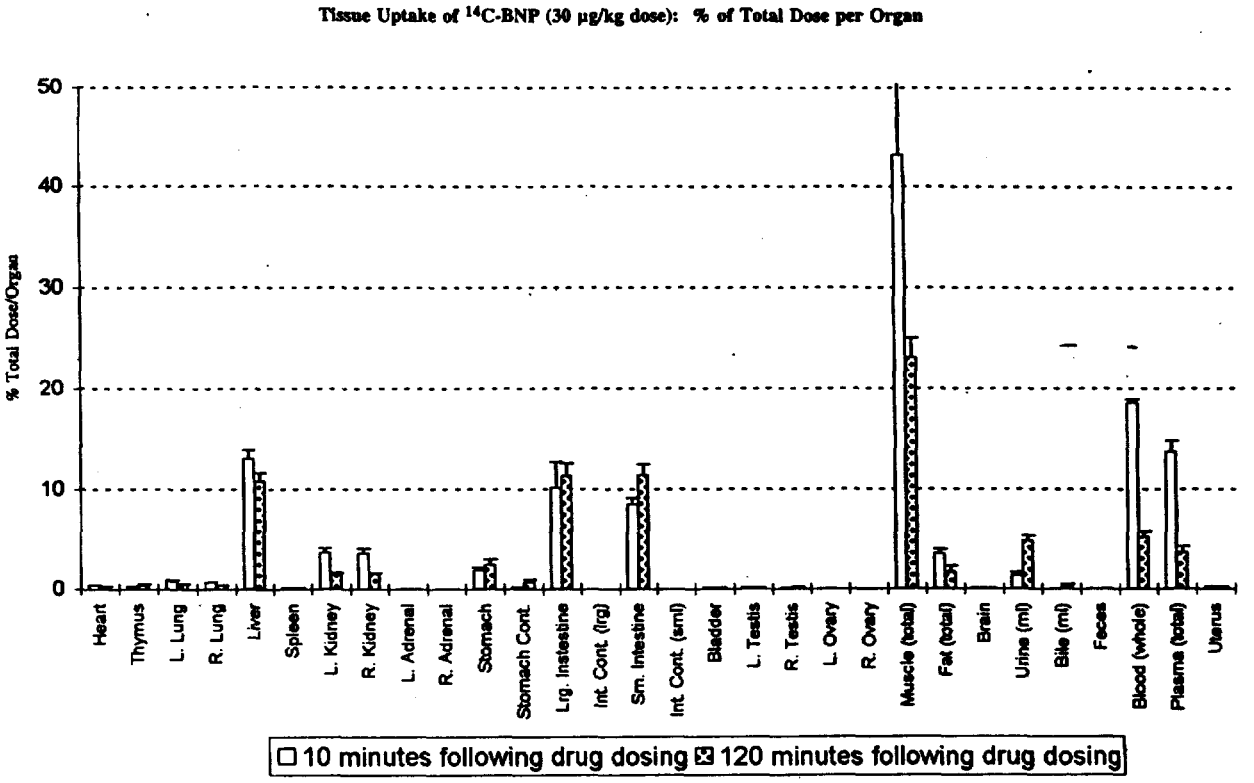
Results: There were no differences in drug tissue distribution between the sexes, and, therefore, the data from males and females were combined and averaged. When expressed as radioactivity uptake per gram of tissue 10 min after injection, the kidneys had the highest amount of label (Figure 52; Sponsor's Figure 2). By 120 min, most of the radioactivity was found in the urine and bile, suggesting that the kidney and liver were important organs for drug clearance.

Figure 52 (Sponsor's Figure 2)



When expressed as percent of total dose per organ 10 or 120 min after injection, skeletal muscle had the highest levels of radioactivity followed by liver and large and small intestine (Figure 53; Sponsor's Figure 3). The high levels in skeletal muscle as a percent of dose probably reflect the relatively large size of the tissue compared to other tissues.

Figure 53 (Sponsor's Figure 3)



Conclusions: The liver and kidneys appeared to be important organs for drug clearance. This was shown by the relatively high levels of radioactivity found in the kidneys, urine, and bile.

5. LABELING (Package Insert):

Carcinogenesis, Mutagenesis, and Impairment of Fertility:

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From:

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LABELING

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6. OVERALL REVIEW AND EVALUATION:

Human B-type natriuretic peptide (hBNP) is a 32- amino acid peptide which is a naturally occurring cardiac hormone produced primarily in the cardiac ventricle and which has vasodilatory, diuretic, natriuretic, and neurohormonal effects. Natrecor (nesiritide) is a preparation containing the purified peptide produced by recombinant DNA technology, with an amino acid sequence identical to the endogenous human BNP cardiac hormone.

Endogenous levels of hBNP are elevated in patients with systolic and diastolic cardiac dysfunction and cardiac hypertrophy. Human BNP binds to the particulate guanylate cyclase receptor (GC- A) of vascular smooth muscle and endothelial cells, leading to increased intracellular concentrations of guanosine 3′5′- cyclic monophosphate (cGMP) which acts as a second messenger to cause smooth muscle cell relaxation and subsequent vasodilation. Venodilation promotes peripheral pooling of blood and decreases venous return to the heart, thereby reducing left ventricular end diastolic pressure and pulmonary capillary wedge pressure (PCWP) (preload). Arterial dilation reduces systemic vascular resistance (afterload) and systemic and pulmonary arterial pressure. Human BNP has also been shown to reduce plasma concentrations of aldosterone in patients and to have mild diuretic and natriuretic properties. The proposed clinical indication is for the short-term intravenous therapy of congestive heart failure (CHF).

6.1. Pharmacology:

In vitro studies designed to measure and compare the induction of cGMP production in response to hBNP in primary bovine vascular smooth muscle cells and primary bovine endothelial cells showed that the hBNP-mediated increase in cGMP in these cells was due to activation of the guanylyl cyclase A (GC-A) cell surface receptor, and that cGMP was likely to be the second messenger mediating most of hBNP's action of vasodilation *in vivo*. This mechanism is similar to nitric oxide's vasodilatory effect in which the nitric oxide-mediated activation of guanylate cyclase and accumulation of cGMP has been shown to be associated with activation of the cGMP-dependent protein kinase. This kinase dephosphorylates specific smooth muscle cell proteins and results in vessel relaxation.

Plasma cGMP levels were used in rabbits as an *in vivo* marker of hBNP receptor activation. After bolus administration, plasma cGMP levels increased in a time- and dose-dependent manner. An approximate half-life for plasma cGMP was derived to be 20.6 ± 5.9 min. These were no significant differences in the cGMP response or in pharmacokinetic parameters after bolus or continuous infusion of either syn-hBNP or rec-hBNP. In rabbits given the high dose of 30 μg/kg syn-hBNP or rec-hBNP, a rapidly metabolized α phase and a more slowly metabolized β phase were detected when compared to the lower dose of 10 μg/kg. There was no difference in the steady-state metabolic clearance rates derived from intravenous continuous infusion of syn-hBNP and rec-hBNP.

In precontracted cultures of human arterial and venous tissue, hBNP showed relaxant activity. This effect was concentration-dependent and was similar to that seen with ANP. hBNP induced statistically significant responses on arterial tissue at ≥ 0.03 nM and on venous tissue at ≥ 1.0 nM. The Sponsor concluded that the therapeutic concentration for hBNP would be about 1 nM, and that this concentration *in vivo* would be expected to exert a vasodilatory effect.

Studies in anesthetized pigs showed that hBNP exerted vasodilatory effects *in vivo*. Coronary blood flow increased in the absence of increases in blood pressure or heart rate. The hBNP-induced increases in vasodilation and coronary blood flow were similar to those seen with nitroglycerin. The vasodilatory effect of hBNP after preconstriction with endothelin-1 suggested to the sponsor a possible treatment during coronary spasm-induced angina or ischemia. Endothelium-derived nitric oxide contributed to hBNP-induced vasodilation in resistance vessels but not in conductance vessels. The vasodilatory effects of hBNP were inhibited by indomethacin and, therefore, were dependent upon prostaglandin synthesis and release. The vasodilatory effects of hBNP were not dependent upon ATP-sensitive potassium channels that normally help regulate coronary vascular tone. The Sponsor speculated that some of the effects of hBNP seen in human patients may in fact be due to increased levels of ANP, since administered hBNP may compete with ANP for metabolism by the natriuretic peptide clearance receptor or the neutral endopeptidase resulting in higher levels of ANP.

Since the rat appeared to be less responsive to hBNP than dogs or baboons, the rabbit was studied as for its suitability as a convenient model for hBNP-induced hemodynamic, cardiac, hormonal, and renal effects. Bolus administration of 3 μg/kg hBNP to rabbits had significant, but transient, diuretic (9-11X increase in urine volume) and natriuretic (11-13X increase in sodium excretion) effects in rabbits. Repeated administration of hBNP 60 min apart did not result in desensitization of the effects. There was a corresponding drop in systolic blood pressure that correlated with the renal diuretic and natriuretic effects. The hypotension reached its nadir about 20 min after each administration, but returned to near baseline levels by 60 min. The Sponsor stated that the effects seen in the rabbit were similar to those seen in dogs, and that the rabbit appeared to be a suitable model to study the pharmacologic actions of hBNP.

Since the previous study showed that hBNP increased urine volume and sodium excretion, and reduced systolic blood pressure in rabbits, a study was conducted using these same endpoints to compare the activities of synthetic (syn) versus recombinant (rec) hBNP in rabbits. Results showed that intravenous bolus administration of hBNP, in either the synthetic or recombinant form, resulted in significant, but transient, diuresis (≥ 3 μg/kg) and natriuresis (≥ 1 μg/kg) without significant kaliuresis in rabbits (up to 10 μg/kg). Systolic blood pressure, pulse pressure, and mean arterial pressure were decreased (≥ 3 μg/kg) and these cardiovascular effects correlated with the renal effects, in that the effects occurred within the first 20 min after dosing and returned to near baseline levels by 60 min. There were no significant differences in renal or cardiovascular effects in response to syn-hBNP versus rec-hBNP administration in rabbits.

Based on previous studies in which hBNP relaxed human artery tissue that had been precontracted with the alpha adrenergic receptor agonist phenylephrine, studies were conducted in rabbits to show that hBNP reduced the norepinephrine-induced increase in blood pressure (acute hypertension) in rabbits, while it had no effect on the norepinephrine-induced drop in heart rate. The Sponsor suggested that hBNP may be useful in controlling blood pressure in the setting of post-surgical hypertension.

Given the reported association between elevated plasma catecholamines, plasma dopamine beta hydroxylase activity, and acute hypertension following surgery in humans, studies were conducted in rabbits with norepinephrine-induced hypertension as a model for examining the use of hBNP for controlling acute hypertension associated with surgical procedures. Continuous infusion of hBNP at 0.1-0.5 μg/kg/min decreased systolic, diastolic, and mean arterial pressures in normotensive rabbits and in rabbits with norepinephrine-induced hypertension. The effect on blood pressure reached a nadir by about 105 minutes, but there

appeared to be a trend for blood pressures to begin to rise thereafter even in the presence of continued drug infusion.

Administration of hBNP to anesthetized rabbits by either the intravenous or subcutaneous routes resulted in decreased systolic and diastolic blood pressures (hypotension), increased urine volume (diuresis), and increased urine sodium excretion (natriuresis). Potassium excretion (kaliuresis) was minimally increased. Subcutaneous administration resulted in prolonged effects, particularly the drop in systolic blood pressure and the enhanced renal output (volume and sodium). AUC analysis showed that about 65% of the hBNP given by the s.c. route was absorbed and active when compared to the i.v. route. These results suggest that mechanisms that may prolong drug clearance or increase the deposition of drug into tissue stores may result in undesirable prolongation of the pharmacodynamic effects.

The pharmacodynamics and pharmacokinetics of recombinant hBNP (rec-hBNP) and synthetic hBNP (syn-hBNP) were compared *in vitro* (cultured cells) and *in vivo* (rabbits). Both rec-hBNP and syn-hBNP induced comparable dose-dependent increases in extracellular cGMP when incubated for 1.5 hours with cells expressing the GC-A receptor. In rabbits, a single bolus injection of either rec-hBNP or syn-hBNP resulted in transient and dose-dependent increases in urine volume, urine sodium, and urine potassium. The activities of the two forms were comparable. Heart rates increased 20-40 bpm, but there were no significant differences after hBNP treatment when compared to vehicle (saline). When given by continuous infusion, both forms caused a comparable dose-dependent increase in plasma cGMP levels that peaked less than 10 min after injection. During a continuous infusion dose escalation protocol, pharmacokinetic values and steady-state plasma hBNP concentrations derived for both recombinant and synthetic hBNP dosing groups were equivalent. The half-life values for rec-hBNP and syn-hBNP derived from animals treated with 3 μg/kg of drug were similar (4.7 $\pm$ 0.2 minutes and 5.2 $\pm$ 0.3 minutes, respectively).

Isolated Langendorff-perfused rabbit hearts were used to examine effects hBNP on cardiac performance by monitoring changes in the rate of contraction, coronary flow, and ventricular contractile function. It was concluded that perfusion of isolated rabbit hearts with hBNP at rates ranging from 0.01 μg/min to 100 μg/min did not appear to produce any direct effects on cardiovascular performance when compared to effects seen with vehicle. As expected, the positive control, dobutamine, produced large increases in +dP/dtmax, -dP/dtmax, and in the rate of contraction.

Since the previous study showed that hBNP did not effect cardiac contractility in an isolated rabbit Langendorff-perfused heart preparation, a study was conducted to assess whether hBNP exerted a direct inotropic effect on explanted human heart tissue. Results showed that hBNP did not have a direct effect on human cardiac muscle contractility (trabecular tension) when compared to two known positive inotropic agents, isoproterenol and dobutamine.

Studies in conscious dogs to assess the effects of hBNP on the sinus node, atrioventricular junction, atrial, and ventricular tissue, and on the inducibility of atrial and ventricular arrhythmias showed that infusion of hBNP at doses up to 0.09 µg/kg/min for one hour resulted in increased plasma cGMP levels, indicating activation of the biological receptor for hBNP *in vivo*. This was accompanied by a fall in arterial blood pressure which was consistent with the expected hemodynamic effects on hBNP. However, heart rates were not increased as might have been expected in response to reflex sympathetic stimulation or vagal withdrawal, nor were there any changes in any of the electrophysiologic (ECG) parameters measured.

Binding of the natriuretic peptide clearance (NP-C) receptor with hBNP has been shown to increase intracellular cAMP concentrations. Increased intracellular concentrations of cAMP or cGMP in platelets inhibit their activation and aggregation, an effect which may be undesirable in patients when hemostasis is critical. Since human platelets express the NP-C receptor, studies were conducted to examine the effects of hBNP on the activation and aggregation of human platelets. hBNP when tested at up to 250 μM did not induce aggregation or activation of human platelets. In addition, it did not enhance ADP-mediated aggregation and activation. In contrast, sodium nitroprusside, a nitric oxide donor that increases intracellular concentrations of cGMP, markedly decreased platelet aggregation in response to ADP (1-4 μM) when compared to that seen with ADP alone. It would have been useful to know the platelet levels of cGMP to determine if the lack of an inhibitory effect by hBNP occurred in spite of increased cGMP levels, and if the enhancing effect of nitroprusside on ADP-mediated platelet aggregation was associated with increased cGMP levels. These data would have helped to determine an association between the expected pharmacologic effect of hBNP on increasing cGMP levels and platelet inhibition. The sponsor suggested that selection of hBNP as a vasodilator and hBNP's lack of platelet inhibitory function may be useful to patients in whom hemostasis is important.

Published studies have shown that the ACE inhibitor captopril increased the half-life and steady-state concentrations of infused porcine BNP in rats. Studies were conducted to examine the effect of purified human ACE on hBNP metabolism *in vitro* and the effect of ACE inhibition with captopril on hBNP metabolism *in vivo* using dogs to determine if hBNP plasma levels may potentially be increased following therapy with ACE inhibitors. Results showed that incubation of hBNP with human ACE *in vitro* did not result in significant ACE-specific degradation of hBNP. *In vivo*, captopril did not change the half-life or other pharmacokinetic parameters of hBNP indicating that hBNP was not a substrate for ACE. This implied that concomitant administration of ACE inhibitors to patients would not alter the pharmacokinetic profile of hBNP. These results were different from previous results in which ACE inhibition was found to decrease the metabolism of porcine BNP in rats. However, the present study was consistent with published clinical results in which captopril treatment of congestive heart failure patients did not increase the circulating levels of endogenous BNP. The sponsor concluded that there was no significant effect of ACE on hBNP metabolism.

Since heparin may be used in patients with congestive heart failure and postsurgical hypertension, studies were conducted to examine whether heparin had any effects on the action of hBNP *in vitro*, and if it had any effects on the action and pharmacokinetics of hBNP *in vivo*. Results showed that heparin at concentrations (100 units/ml) up to 50X those found in patients following a typical anticoagulant dose of heparin (2 units/ml) did not inhibit the hBNP-induced release of cGMP from CHO cells *in vitro*. In rabbits, heparin at doses up to 15,000 units/kg, which equals a circulating concentration of 625 units/ml, resulted in no observable decrease on the renal and hemodynamic effects of hBNP. The general lack of an effect of heparin on hBNP-mediated responses in rabbits was inconsistent with a published report (Wei *et al.*, 1987) in which heparin at 700 units/kg inhibited the renal and hemodynamic actions of rat ANP in rats. Possible explanations for the inconsistency were offered including species differences, different heparin binding properties between rat ANP and human BNP, and chemical grade of the heparin used.

6.2. Toxicology:

Acute toxicity studies were conducted in rats and monkeys. In rats, a single i.v. injection of SC-70400 at doses up to 3,000 μg/kg produced no evidence of toxicity when assessed by gross examination after necropsy. In monkeys given up to 500 μg/kg hBNP, there were no drug-related effects on clinical signs, body weight, food consumption, or physical exam data. The pathology report did not indicate any drug-related effects that could be distinguished from the untreated monkeys. Most macroscopic findings in monkeys were apparently secondary to parasitic infections.

In two week toxicity studies in rats, continuous intravenous infusion of SC-70400 at doses up to 20 μg/kg/min for two weeks did not result in histopathological changes indicative of systemic toxicity. Changes in urinalysis parameters, such as decreased urine output and increased urine concentration of electrolytes, were consistent with the known pharmacological properties of the drug as a natriuretic and diuretic agent. No difference in this effect could be distinguished between the low dose of 5 μg/kg/min with the effect seen at the high dose of 20 μg/kg/min. The seemingly paradoxical effect of decreased urine output after two weeks of therapy may have reflected "compensatory homeostatic mechanisms" (such as increased levels of aldosterone or antidiuretic hormone) that were activated early during the course of treatment to conserve fluids and electrolytes, even though ANP is known to inhibit aldosterone secretion. More frequent urine collections could have confirmed this. No clear explanations were offered for the decreased heart and increased kidney weights. However, both organs are sensitive and respond to hemodynamic changes which may occur during the course of treatment with diuretic and natriuretic compounds. There was no evidence of microscopic changes in these organs to indicate cellular alterations. All alterations in urine, heart and kidney weights were reversible after two weeks of recovery. Pharmacokinetics were not evaluated. A no-observable-adverse-effect level (NOAEL) could not be determined due to the changes in urinalysis parameters and decreased heart weights seen at the low dose of 5 μg/kg/min. However, these changes were reversible and, except for decreased heart weight, may have been due to the known pharmacologic effects of SC-70400.

In two week toxicity studies in monkeys, continuous intravenous infusion of SC-70400 at doses from 0.3 to 3.0 μg/kg/min for two weeks resulted in minimal adverse effects. Body weights were minimally affected (5-6% weight loss vs 1-2% for controls). There were no drug-related effects during physical (respiration rates and rectal temperatures) or ophthalmic examination, and there were no drug-related effects on surface ECG measurements. Systolic, diastolic, and mean arterial blood pressures were reduced equally (approximately 20-25% when compared to untreated controls) at all doses beginning on Day 3 (first time point measured) in both males and females, an effect consistent with the relaxant or vasodilatory activity seen in precontracted cultures of human arterial and venous tissue. SC-70400 did not significantly alter sodium and chloride excretion and urine volume in spite of the drug's known natriuretic and diuretic effects. This may reflect, however, the anti-diuretic effect of hBNP-mediated hypotension. Except for the slightly reduced heart-to-body weight ratios in males, there were no other drug-related effects on organ weights. No microscopic findings related to drug-treatment were found. Plasma BNP concentrations showed dose-proportionality and were relatively stable over the two week infusion period. The high dose of 3.0 μg/kg/min represented a 200X multiple of the recommended human dose of 0.015 μg/kg/min. The steady state concentrations (postdose plasma hBNP concentrations minus predose hBNP plasma concentrations) achieved in monkeys

at the high dose of 3.0 μg/kg/min (50.6 ng/ml) after 6 hours of continuous infusion were 24X the steady state concentrations (2.1 ng/ml) achieved in humans at the recommended dose of 0.015 μg/kg/min after 6 hours of continuous infusion (Study No. 704.311).

An additional two week continuous intravenous infusion toxicity study was conducted in monkeys to assess and compare the toxicity of recombinant versus synthetic hBNP. Also assessed were reversibility of effects of recombinant hBNP after a 2-week recovery period, the hemolytic potential or recombinant hBNP for monkey and human whole blood, and compatibility of recombinant hBNP for monkey and human serum and plasma. Results showed that there were no remarkable clinical findings that could be attributed to drug treatment. Changes, such as hypoactivity, occurred with equal frequency across treatment groups. Some of the animals were anemic, but this was thought to be due to the frequent blood collections and reduced erythropoiesis secondary to inflammation associated with chronic catheterization. hBNP reduced mean arterial blood pressures (MAP) equally across all doses (0.3-3.0 μg/kg/min). Blood pressures after two weeks of recovery remained below those of untreated controls. There were no significant differences on MAP between syn-hBNP and rec-hBNP when given at 1.0 μg/kg/min. Urine sodium excretion was lower in drug-treated animals by Week 1. No explanation was offered to account for the effects given the known natriuretic activity of hBNP, although hBNP-mediated hypotension and decreased renal perfusion may be involved. The effect on lowering sodium excretion was absent after two weeks of recovery. There were some changes in organ-to-body weight ratios. These included lower heart weight ratios in drug-treated males and higher liver weight ratios in drug-treated females. Lower heart-to-body weight ratios were also seen in a previous two-week monkey toxicity study using syn-hBNP, and were attributed to decreased blood pressures. However, there were no macroscopic or microscopic changes in any of the organs to indicate drug-induced toxicity. Pharmacokinetic analyses showed that steady-state and dose-related hBNP plasma concentrations occurred within the first 6 hours of infusion. There was no accumulation of hBNP in plasma over time (6 hours to 15 days). There were essentially no differences in hBNP plasma concentrations between similar doses (1.0 μg/kg/min) of rec-hBNP and syn-hBNP. Additional studies showed that rec-hBNP did not cause hemolysis when mixed with whole monkey or human blood, and rec-hBNP did not cause precipitation or coagulation when mixed with monkey or human plasma or serum.

Studies were conducted in rabbits to assess the local tolerance of the SC-70400 (hBNP) when administered by 1 hour infusions to rabbits as a single dose or as multiple doses. Results showed that hemorrhage and chronic inflammation were found in both control and drug-treated groups, and were attributed to mechanical trauma, and not to drug or vehicle treatment. It was concluded that there was no local irritation in the infusion sites of rabbits given SC-70400 at doses up to 200 μg/kg infused over one hour each day for up to 5 consecutive days.

Additional studies were conducted in rabbits to determine if anti-hBNP antibodies were generated in rabbits after repeated exposure to either recombinant hBNP (rec-hBNP) or synthetic hBNP (syn-hBNP). Results showed that there was no measurable antibody response in any of the serum samples collected from rabbits prior to or following repeated administration with synthetic or recombinant hBNP. These results indicated that hBNP was not immunogenic in rabbits, and that the development of antibodies in humans, which can bind drug and reduce effectiveness, is not likely to occur.

The hemolytic potential of recombinant hBNP for monkey and human whole blood was examined, as well as the compatibility of recombinant hBNP for monkey and human serum and plasma. Results showed that rec-hBNP at a final concentration of 30 μg/ml did not cause

hemolysis when mixed with whole monkey or human blood, and it did not cause precipitation or coagulation when mixed with monkey or human plasma or serum at the same final concentration.

Since recombinant human BNP has an identical amino acid sequence to the endogenous peptide found in humans, only a single study was conducted to examine the potential mutagenicity of hBNP, the Ames test. Results showed that recombinant human BNP did not cause a positive increase in the number of revertants in any of the bacterial tester strains either in the presence or absence of metabolic activation (±S9). However, minimally positive results (1.9-2.0-fold increase in revertants) were found with the *S. typhimurium* tester strain TA98 in the preincubation exposure method. When the method was modified to remove exposure to the test article during the growth phase of the assay (a published treat and plate exposure method; Green and Muriel, 1976), no positive results were found.

No carcinogenicity or reproductive toxicology studies were conducted with Natrecor.

6.3. Absorption, Distribution, Metabolism, and Elimination (ADME):

Human BNP binds and activates the guanylyl cyclase-A (GC-A) membrane receptor resulting in synthesis and intracellular accumulation of cyclic GMP (cGMP), much of which is released from the cell. Administration of hBNP to dogs and humans has been shown to result in increased plasma concentrations of cGMP. A study was conducted to compare the ability of synthetic (syn) and recombinant (rec) hBNP to increase the plasma concentration of cyclic GMP after bolus and continuous infusion into rabbits. Results showed that plasma cGMP concentrations were increased in a time and dose-dependent manner, an indication of its *in vivo* pharmacologic activity of guanylyl cylcase A receptor activation. Following bolus administration, plasma cGMP levels peaked within 15 min of administration and returned to baseline within 30-60 min with an approximate half-life of 20.6 min for cGMP. The pharmacokinetic parameters and levels of plasma cyclic GMP following bolus or continuous I.V. administration of syn-hBNP and rec-hBNP were similar.

The previous study, in which certain pharmacological properties and equivalence of rec-hBNP and syn-hBNP were measured in rabbits, was expanded to include hBNP-induced activation of the human biological receptor (GC-A) in tissue culture, renal effects of bolus hBNP administration in anesthetized rabbits, and cardiovascular effects of bolus hBNP administration in anesthetized rabbits. Results *in vitro* showed that levels of cGMP released from CHO cells expressing the human GC-A receptor were similar after treatment with either syn-hBNP or rec-hBNP. In anesthetized rabbits, intravenous bolus administration of increasing doses of rec-hBNP or syn-hBNP resulted in transient and dose-dependent increases in urine volume, urine sodium excretion rate, and urine potassium excretion rate, and in transient decreases in systolic, diastolic, and mean arterial blood pressures. There were no significant differences in renal or cardiovascular effects between syn-hBNP and rec-hBNP. In conscious rabbits, continuous or bolus infusion rec-hBNP and syn-hBNP resulted in dose-dependent increases in plasma cyclic GMP. Plasma hBNP values were fit to a two compartment model which assumed that drug concentrations declined exponentially as the sum of two first-order processes. The half-life values for the α phase ($t1/2\alpha$, 3.7 minutes and 3.3 minutes) and β phase ($t1/2\beta$, 15.0 minutes and 15.5 minutes) derived for rec-hBNP and syn-hBNP, respectively, were equivalent, as were half-life values, micro-rate constants, volumes of distribution, and clearance. Steady-state

plasma hBNP concentrations derived for both recombinant and synthetic hBNP dosing groups were equivalent for each of the three doses tested.

Studies were conducted in anesthetized rabbits to examine the cardiovascular (blood pressures and heart rates) and renal (urine volume and sodium excretion) effects of recombinant hBNP administered by bolus subcutaneous and intravenous injection. Pharmacokinetic parameters were also determined. Results showed that both intravenous and subcutaneous administration of recombinant hBNP to rabbits resulted in decreased systolic and diastolic blood pressures, increased heart rates, and increased urine volume and urine sodium excretion. Effects on urine potassium excretion were not as marked. hBNP delivered by the subcutaneous route was biologically active. The vascular and renal effects were more prolonged following subcutaneous administration, due to slower clearance. The loss of hBNP from the plasma compartment was fit to a two-compartment (α and β) model in rabbit, dog, and human with similar half lives.

Studies were conducted to examine the effect of human ACE on hBNP metabolism *in vitro*, and the effect of captopril on hBNP metabolism *in vivo* (dogs). This was performed because of published studies in which it was shown that an ACE inhibitor, captopril, increased the half-life and steady-state concentrations of infused porcine BNP in rats (Vanneste Y. *et al.*, 1990), and in which captopril potentiated the half-life of porcine BNP in rat serum and protected the peptide from degradation by a rabbit lung preparation of ACE. Results of the *in vitro* studies showed that human ACE did not degrade hBNP after 4 hours of incubation, and after 16 hours there was some degradation, but was not ACE-specific. Also, hBNP was more resistant to neutral endopeptidase 24.11-mediated degradation than was ANP. Studies *in vivo* showed that captopril pretreatment had no effect on hBNP half-life (alpha or beta), micro rate constants, Vc , Vdss , and CL in dogs. The differences in results between those previously reported by Vanneste Y. *et al* (1990), in which captopril increased the half-life and steady-state concentrations of infused porcine BNP in rats, and those reported here, in which captopril had no effect on hBNP half-life in dogs, may have been due to the different species of BNP and ACE used in the two studies.

A pharmacokinetic study was conducted in monkeys in which hBNP was administered as a two-hour continuous i.v. infusion at doses up to 1.0 µg/kg/min. Results showed that BNP was cleared from the plasma relatively rapidly with a t1/2α of 2.7 ± 0.6 min and a t1/2β of 26.3 ± 13.4 min. Clearance (CL) values decreased with increasing dose while volume of distribution at steady-state (Vss) values were similar at all doses.

A study was conducted to examine the elimination of hBNP from plasma in rabbits. Natriuretic peptide clearance (NP-C) receptors and neutral endopeptidase (NEP) are apparently abundantly expressed in the kidney, and the kidney may be involved in BNP clearance by metabolism or by filtration. The NP-C receptor mediates the cellular internalization and subsequent lysosomal degradation of ANP and possibly BNP, and BNP has been shown to be a substrate for NEP. This study used restricted blood flow to the kidney, blockade of the natriuretic peptide clearance (NP-C) receptor with a specific agonist, and inhibition of neutral endopeptidase (NEP) to examine the role of the kidney, the NP-C receptor, and NEP digestion, respectively, on the elimination of hBNP from plasma in rabbits. Also, *in vitro* studies examined whether hBNP was a substrate for human NEP. Results showed that several pathways were involved in the elimination of hBNP from plasma in rabbits. These included: (1) elimination by renal filtration or proteolysis (ligation of the renal arteries to restrict renal blood flow resulted in increased mean steady-state plasma hBNP concentrations; (2) elimination by natriuretic peptide

clearance (NP-C) receptor-mediated uptake (co-infusion of the natriuretic peptide clearance receptor agonist C-ANP reduced receptor binding, internalization, and subsequent lysosomal degradation); and (3) elimination by neutral endopeptidase (NEP)-mediated proteolysis (co-infusion of the NEP-specific inhibitor phosphoramidon reduced proteolysis). *In vitro* studies showed that hBNP was more resistant to peptidase digestion than hANP.

A study was conducted in rabbits to determine the tissue distribution of radiolabeled synthetic hBNP when administered as a bolus intravenous injection. Results showed that there were no differences in drug tissue distribution between the sexes. Also, the liver and kidneys appeared to be important organs for drug clearance. This was shown by the relatively high levels of radioactivity found in the kidneys, urine, and bile. When expressed as radioactivity uptake per gram of tissue 10 min after injection, the kidneys had the highest amount of label. By 120 min, most of the radioactivity was found in the urine and bile.

6.4. Conclusions:

Preclinical studies both *in vitro* and *in vivo* showed that human BNP (hBNP) binds to and activates the guanylyl cyclase A (GC-A) cell surface receptor on vascular smooth muscle cells. This results in increased intracellular levels of the second messenger cyclic GMP and leads to reduced blood pressure due to vasodilation of both venous and arterial vessels. Additionally, hBNP had significant diuretic and natriuretic effects which may have been mediated through changes in the renin-angiotensin-aldosterone system, similar to that known for atrial natriuretic peptide (ANP). ANP is known to suppress renin-angiotensin-mediated vasoconstriction and aldosterone-mediated sodium and fluid retention. However, this pathway was not adequately explored in the preclinical studies. Also, the sponsor did not perform studies to examine the relative diuretic versus vasodilatory potency *in vivo*. The known effect of the natriuretic peptides to suppress aldosterone secretion appears to be contrary to the "paradoxical" effect of possible increased aldosterone secretion as suggested by the sponsor in which urine sodium and volume excretion were reduced after prolonged (two weeks) continuous infusion of hBNP.

Toxicity studies of up to two weeks duration in rats and monkeys showed no evidence of drug-induced toxicity that could not be accounted for by the known pharmacologic effects of the drug. Decreased blood pressure was the main effect, a finding that was consistent with the drug's relaxant or vasodilatory activity. Heart and kidney weights showed some decreases that were reversible, but there was no evidence of microscopic changes in these organs to indicate cellular alterations. In the two week monkey toxicity studies, the systemic plasma exposures at the high dose of 3.0 µg/kg/min were 24X that achieved in humans at the recommended dose of 0.015 g/kg/min indicating an adequate margin of safety.

Elimination of hBNP occurs by several pathways including renal filtration or proteolysis, natriuretic peptide clearance (NP-C) receptor-mediated uptake, and neutral endopeptidase (NEP)-mediated proteolysis. No differences in pharmacology or toxicology were found between synthetic and recombinant hBNP.

Finally, there is some evidence from the clinical trials (see Medical Officer's review) to suggest that drug-induced decreases in pulmonary capillary wedge pressures (PCWP) begin to diminish in the continued presence of drug infusion. Studies conducted in conscious rabbits with norepinephrine-induced hypotension showed a slight trend for mean arterial blood pressures to rise after reaching a nadir even in the presence of continued drug infusion. However, blood pressures appeared to be rising in the control rabbits as well, and it is difficult to determine if this

effect was due to drug tolerance or tachyphylaxis. Mechanistically, down-regulation of ANP receptors on vascular cells has been reported in cell culture studies and in patients with heart failure, indicating that prolonged elevation of circulating ANP or BNP levels may lead to reduced drug responsiveness (see Appendix below). This possibility may need to be addressed by the sponsor since it may have some bearing on the recommended drug regimen.

NDA #20-920

7. RECOMMENDATIONS:

Based on the animal safety studies reviewed above, it is recommended that Natrecor® (NDA #20-920) be approvable with the suggested change in the labeling (see page 97).

/S/ 12/8/98

Thomas Papoian, Ph.D.
Pharmacologist

Concur: /S/ 12/8/98

cc:
Orig. NDA
HFD-110
HFD-110/ D. Willard
HFD-110/ T. Papoian
HFD-024/J. DeGeorge
HFD-345 (Scientific Invest.)

8. APPENDIX:

Evidence from the clinical trials, particularly Study No. 704.311, suggested that drug-induced decreases in pulmonary capillary wedge pressures (PCWP), which reached a nadir by 6 hours, began to rise thereafter even in the continued presence of drug infusion (see Medical Officer's review). Studies in rabbits showed similar effects on mean arterial pressure, but the results were not as clear, due possibly to the shorter 2 hour infusion time used in rabbits (see Section 2.8, Study No. 00238, Figure 12, above) versus the 24 hour infusion time used in patients.

However, the possibility exists that the diminishing effect seen in patients may have been due to induction of drug tolerance or tachyphylaxis. Since reduced patient responsiveness over the course of treatment may have bearing on drug effectiveness, an attempt was made to briefly summarize selected published studies that explored possible mechanisms of tolerance to another natriuretic peptide, ANP. Results conducted (1) in cultured vascular cells, (2) in rats, and (3) in patients with congestive heart failure (CHF) indicated that prolonged exposure to elevated levels of ANP were associated with ANP-receptor down-regulation and reduced cyclic GMP production. By extension, similar mechanisms may also be involved with BNP treatment.

1. Cell Culture Studies:

Several groups have demonstrated ANP-mediated down-regulation of its receptors in cultured vascular smooth muscle cells (VSMC). Using smooth muscle cells isolated from rat mesenteric artery, Schiffrin *et al.* (1986) showed that exposure of cells to 10^{-8} M ANF for up to 2 hours did not change the density of binding sites. After 24 hours, however, the density of binding sites decreased 40%. There was a recovery in the density of binding sites when the cells were washed and cultured in the absence of ANP for an additional 24 hours.

Using rat aortic VSMC, Hirata *et al.* (1985) showed that treatment of cells for 24 hours with human ANP at 3.2×10^{-9} and 3.2×10^{-8} M resulted in a 55% and 74% decrease, respectively, in the number ANP binding sites per cell. Subsequent studies (Hirata *et al.* 1986) showed that 70-75% of the receptor loss occurred after 4 hours of treatment, and that recovery of receptors did not occur in the presence of protein synthesis inhibitors.

Studies to determine if ANP receptor down-regulation was associated with a reduced cyclic GMP response (desensitization) have been inconsistent. Using rat VSMC, Hirata *et al.* (1987) showed that treatment of cells with 1.6×10^{-7} M hANP for 24 hours resulted in a marked reduction (~80%) of the ANP receptor number. After removal of the bound hANP, the cells were further treated with hANP for 10 min and the intracellular cGMP response measured. Results showed that intracellular cGMP levels rose to the same extent in pretreated (down-regulated) cells as in control (non-pretreated) cells, indicating that reduced receptor density was not associated with reduced cell responsiveness. The authors suggested that ANP receptors are heterologous, and ANP preferentially down-regulates an ANP receptor subtype that is not coupled to G-cyclase.

Another group of investigators did show that ANP receptor down-regulation was associated with a desensitization of ANP-induced cGMP production. Using rat VSMC, Roubert *et al.* (1987) demonstrated 7%, 47%, and 55% down-regulation of ANP receptors in cells after treatment with 10^{-9}, 10^{-8}, and 10^{-7} M rat ANP, respectively. However, in contrast to the results reported by Hirata *et al.* (1987), cGMP production induced by rANP (10^{-7} M) was decreased

20%, 38%, and 65% in cells pretreated (down-regulated) with rANP (10^{-9}, 10^{-8}, and 10^{-7} M, respectively), suggesting desensitization of ANP-mediated cGMP production.

ANP-responsive cells possess two types of ANP receptors. The B-type or biologically active receptor triggers the rise in intracellular cGMP and the subsequent vasodilatory actions of ANP. The C-type or clearance receptor constitutes up to 95% of the ANP receptors, is not coupled to guanylate cyclase, and is responsible for uptake and clearance of ANP from the circulation. Using primary cultures of guinea pig coronary artery smooth muscle cells (CASM) and thoracic aorta smooth muscle cells (TASM), Zhang *et al.* showed that exposure of TASM, which express both the B- and C-receptors, to ANP (10^{-6} M) for 24 hours resulted in down-regulation of the C-receptor, whereas exposure of CASM, which express only the C-receptor, to ANP did not alter ANP binding. Further, activation of guanylyl cyclase with sodium nitroprusside in both cell types resulted in decreased ANP receptor binding. These results suggested to the authors that ANP activation of the B-receptor, with subsequent elevations in intracellular cGMP levels, was necessary for down-regulation of the C-receptor. Reduced numbers of C-receptors by blocking with a selective agonist have been shown in the rat to result in elevated ANP levels and increased diuresis, suggesting a negative feedback mechanism for maintaining circulating ANP levels (Maack *et al.* 1987).

With regards to BNP, Hirata *et al.* (1988) showed that porcine BNP and human ANP interact with the same receptor sites on cultured rat VSMC to activate guanlylate cyclase. The binding capacity and affinity of BNP appeared to be identical to that of ANP. The authors suggested that long-term exposure to BNP may also induce homologous receptor down-regulation, and the existence of a unique receptor for BNP distinct from ANP on other target tissues was considered.

2. Studies in Rats:

Using an experimental model of volume-expanded hypertension, the deoxycorticosterone acetate (DOCA)-salt rat, Schiffrin and St-Louis (1987) examined vascular ANP binding sites, responses to ANP of norepinephrine (NE)-precontracted aorta, and the hypotensive action of ANP infused *in vivo* into DOCA-salt hypertensive rats. Uninephrectomized rats served as controls. Results showed that the density of mesenteric vascular ANP binding sites was decreased 70% in DOCA-salt hypertensive rats when compared to uninephrectomized control rats. Further, the sensitivity of (NE)-precontracted vascular strips to ANP from DOCA-salt hypertensive rats was reduced. Infusion of ANP at 100-300 ng/hr for 4 days into DOCA-salt hypertensive rats did not result in lowering of blood pressure, whereas blood pressure was significantly reduced in the uninephrectomized controls. ANP plasma concentrations on DOCA-salt hypertensive rats was increased nearly 7-fold when compared to controls. These results suggested to the authors that raised ANP plasma concentrations in DOCA-salt hypertensive rats were associated with a decreased density of ANP receptors in the mesenteric vascular beds, a decreased sensitivity of vascular responses to ANP *in vitro*, and a resistance to the blood pressure-lowering action of ANP *in vivo*.

3. Studies in Patients with Congestive Heart Failure (CHF):

ANP binding sites on platelets were used by Schiffrin (1988) to examine the association between elevated circulating ANP levels and platelet ANP receptor density in patients with

severe (NYHA class III-IV) CHF. Results showed that CHF patients had a 6-fold increase in plasma ANP levels when compared to patients without cardiac disease. The increases in ANP levels in CHF patients were associated with a 47% decrease in the density of platelet ANP binding sites. It was not determined in these patients if the reduced density of ANP binding sites on platelets was associated with a reduced ANP responsiveness.

In a study of patients with varying degrees of heart failure, Tsutamoto *et al.* (1993) correlated plasma ANP levels with cGMP levels in peripheral vascular beds (femoral artery and femoral vein). Results showed that in patients with mild CHF, the plasma ANP levels correlated with the plasma cGMP levels from femoral artery and vein. However, in patients with severe CHF, plasma cGMP levels reached a plateau despite high levels of plasma ANP. Administration of exogenous ANP to patients with acute severe CHF and to patients with mild CHF resulted in increased plasma cGMP levels, while the cGMP response to exogenously administered ANP in patients with severe CHF was attenuated. The authors concluded that endogenous ANP in mild heart failure may result in vasodilation and reduction of the afterload. However, in patients with severe heart failure, where high ANP levels are chronically sustained, down-regulation of ANP receptors may occur, resulting in negation of the compensatory effects of ANP. Although not addressed by the authors, similar effects could be expected with exogenously administered BNP. The same authors (Takayoshi *et al.* 1992) also reported similar correlations of ANP to cGMP levels in the pulmonary vascular beds in patients with mild and severe CHF.

Conclusions:

Studies in cultures of VSMC, in DOCA-salt hypertensive rats, and in patients with mild to severe CHF have shown that chronic exposure to elevated levels of ANP is associated with down regulation of ANP receptors (also termed guanylate cyclase or GC-A receptors) which may occur within a matter of hours. The decreased density of ANP receptors was associated with desensitization or attenuation of the cGMP or vascular responses, although the results from the *in vitro* studies were inconsistent. Overall, these results implied that administration of exogenous hANP, and by extension hBNP, to patients with mild CHF may result in an initial vasodilatory effect. However, sustained exposure may lead to reduced pharmacologic activity through receptor down-regulation. In severe CHF patients whose GC-A receptors are already down-regulated, further administration of hBNP may be of limited utility.

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