Pharmacokinetics and Biological Actions of Subcutaneously Administered Human Brain Natriuretic Peptide

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Accepted for publication May 26, 1998 This paper is available online at http://www.jpet.org

ABSTRACT
Human brain natriuretic peptide (hBNP) has demonstrated favorable hemodynamic effects in patients with congestive heart failure; however, the peptidic nature of this compound has focused clinical testing on protocols involving intravenous delivery. We have studied subcutaneous delivery as an alternative method of administering hBNP. Administration of 30 μg/kg hBNP by either subcutaneous or intravenous delivery protocols resulted in significant hBNP-immunoreactive material in the plasma with area under the plasma concentration-time curve values of 310 ± 20 nmol×mins/liter and 187 ± 47 nmol×mins/liter, respectively. Plasma cyclic GMP, a surrogate marker of activation of the biological receptor for hBNP, was elevated for a longer period of time following subcutaneous delivery compared with intravenous delivery. Subcutaneous delivery of 30 μg/kg hBNP resulted in natriuresis, diuresis and reduced systolic blood pressure in anesthetized normotensive rabbits, effects similar in magnitude yet prolonged in duration compared with those elicited by the same dose of hBNP delivered intravenously. Systolic blood pressure following hBNP treatment remained below base-line values for 50 and 150 min following intravenous and subcutaneous delivery protocols, respectively. These results suggest that subcutaneous delivery of hBNP may be a viable therapeutic alternative to intravenous modes of delivery.

Human brain natriuretic peptide (hBNP) is a 32-amino-acid, cardiac-derived peptide hormone with potent cardiovascular and renal actions (Lewicki andProtter, 1995). Studies have shown that hBNP is a vasodilator (Protter et al., 1996) that reduces cardiac filling pressures and produces a diuretic and natriuretic response (Clavell et al., 1993; Clemens et al., 1997). The biological properties of this hormone have prompted testing its potential therapeutic effects in patients with congestive heart failure and hypertension, and beneficial effects have recently been demonstrated. Intravenous administration of hBNP to patients with congestive heart failure results in a decrease in pulmonary capillary wedge pressure and an increase in cardiac output (Hobbs et al., 1996; Marcus et al., 1995; Yoshimura et al., 1991). The peptidic nature of hBNP (fig. 1) has focused preclinical and clinical studies on protocols using intravenous modes of delivery that are relatively short in duration. Subcutaneous delivery of hBNP may offer considerable advantages over intravenous protocols in certain clinical settings, particularly those involving prolonged treatment. The effectiveness of subcutaneous delivery of hBNP has not been tested in animals or humans.

In rabbits, intravenous hBNP elevates plasma cyclic GMP (Clemens et al., 1997), consistent with activation of the membrane-bound guanylyl cyclase-A receptor for which hBNP has been shown to be a potent ligand (Schoenfeld et al., 1995). In addition, hBNP reduces blood pressure and stimulates diuresis and natriuresis when administered intravenously to anesthetized rabbits (Clemens et al., 1997). In the studies reported here, we use these end points to compare the biological actions of hBNP administered to rabbits by intravenous and subcutaneous delivery protocols. In addition, the plasma hBNP levels achieved with these two delivery protocols were estimated with an hBNP-specific immunoassay. Subcutaneous administration was found to be a surprisingly efficient method of delivering the peptide to the vascular space in a biologically active form.

Materials and Methods

Animals. Male New Zealand White rabbits (1.5–2.0 kg), purchased from R&R Rabbitry (Stanwood, WA), were housed individually for at least 1 week prior to study and allowed food and water ad libitum.

Materials. Recombinant hBNP was expressed in bacteria and purified to >95% homogeneity. The purity and identity of the peptide were assessed by reverse phase liquid chromatography, amino acid sequence analysis and amino acid composition (data not shown). Endotoxin levels, determined by a limulus amebocyte lysate assay, was less than or equal to 5 endotoxin units per milligram (data not shown). Pentobarbital was purchased from Anpro Pharmaceuticals.
plasma (Pel-Freez; Rogers, AR). Rabbit test samples were diluted into normal pooled rabbit plasma, and 0.1 ml of either test sample or reference sample was added to appropriate microtiter wells. The samples were incubated overnight at 4°C on an orbital shaker and then rinsed once with wash buffer. Biotinylated hBNP (125 pg/well in 0.2 ml wash buffer with 1% BSA) was added and then incubated for 1 hr at 4°C. Supernatant was removed, and streptavidin-horse-radish peroxidase (0.2 ml 1:3000 dilution in wash buffer with 1% BSA) was added for 30 min at 4°C. The samples were then rinsed five times with wash buffer, after which trimethylbenzidine substrate (0.2 ml/well) was added and incubated at room temperature for 30 mins. The reaction was stopped with 2.5 N sulfuric acid and optical density at 450 nm was determined using a Spectramax 250 (Molecular Devices; Sunnyvale, CA). This assay has a working range of 40 to 400 pg/ml (CV <12%).

**Plasma cyclic GMP determinations.** Plasma cyclic GMP levels were determined by radioimmunoassay. The labeled antigen was a succinyl tyrosine-(2,5)-methyl ester derivative of cyclic GMP. Separation of bound cyclic GMP from free antigen was achieved by the use of a prereacted primary and secondary antibody complex. Prior to the assay, the plasma samples were extracted with ethanol, and the supernatants were evaporated to dryness in a Speed Vac concentrator (Savant Instruments, Holbrook, NY). The dried samples were reconstituted with sodium acetate buffer prepared according to the manufacturer's instructions. Plasma cyclic GMP levels were determined by interpolation from the standard curve. Any sample with levels above the range of the assay (10 pmol/ml) was diluted appropriately and reassayed. The lowest level of detection was 0.01 pmol/ml. Interassay and intra-assay coefficients of variation were 6.8% and 10.4%, respectively.

**Data analysis.** Area under the plasma hBNP concentration-time curve was determined using the integrate-area function in Kaleidagraph v3.0.4 (Synergy Software, Reading, PA). Plasma hBNP values resulting from intravenous treatment were best fitted to a two-compartment model assuming drug concentrations decline biexponentially as the sum of two first-order processes using the formula: 
\[ C_2 = A \exp(-\alpha t) + B \exp(-\beta t). \]
Plasma hBNP values resulting from subcutaneous treatment were best fitted to a one-compartment model assuming drug concentrations decline exponentially using the formula: 
\[ C_1 = A \exp(-\omega t). \]
Values for \(t_{1/2a}\) and \(t_{1/2b}\) were calculated from 0.693/\(a\) and 0.693/\(b\), respectively.

In the intravenous and subcutaneous administration groups, plasma cyclic GMP values obtained form each time point following hBNP treatment were compared with base-line, pretreatment values within each group by repeated measures analysis of variance using the Dunnett multiple comparisons post-test. In addition, plasma cyclic GMP values at each time point following subcutaneous hBNP treatment were compared with plasma cyclic GMP values obtained at the same time points in the intravenous hBNP group by unpaired, two-tailed \(t\) test. A value of \(P < .05\) was considered significant.

Data from each 10-min hemodynamic and 20-min renal collection period were averaged and expressed as the mean ± S.E.M. Data obtained from the intravenous hBNP and subcutaneous hBNP groups were compared with data obtained from vehicle treated group by analysis of variance using the Dunnett multiple comparisons post-test. As blood pressures in the vehicle-treated group tended to rise during the course of the experiment, changes in systolic and diastolic blood pressures were also compared with base-line values by repeated measures analysis of variance using Dunnett multiple comparisons post-test. A value of \(P < .05\) was considered significant.

**Results**

**Plasma hBNP levels resulting from subcutaneous and intravenous hBNP delivery protocols.** Administration of hBNP by either intravenous or subcutaneous delivery protocols resulted in significant levels of hBNP-immunoreac-
tive material in the plasma (see fig. 2; peak values of 26.6 ± 4.3 and 2.3 ± 0.6 nmol/liter were achieved by the intravenous and subcutaneous protocols, respectively). Values for plasma hBNP-immunoreactive material prior to hBNP administration were less than 0.012 nmol/liter. The calculated area under the plasma concentration-time curve (180 min) for hBNP delivered by the intravenous and subcutaneous routes were 310 ± 20 and 187 ± 47 nmol×mins/liter, respectively. The plasma decay curves for hBNP administered by the intravenous protocol were best fitted to a two-compartment model with computed t₁/₂α = 5.5 ± 0.9 min and t₁/₂β = 27.4 ± 9.7 mins. Plasma hBNP-immunoreactive material following subcutaneous hBNP administration achieved a maximum level between 15 and 30 min following treatment and then declined exponentially (best fit to a one-compartment model) with a t₁/₂ of 28.7 ± 2.4 min.

**Plasma cyclic GMP levels resulting from subcutaneous and intravenous hBNP delivery protocols.** Bolus administration of 30 μg/kg hBNP by either intravenous or subcutaneous delivery protocols resulted in a time-related increase in plasma cyclic GMP (fig. 3). Following subcutaneous hBNP treatment, plasma cyclic GMP levels were maximally elevated within 20 min and remained elevated for 60 min. Following intravenous hBNP administration, plasma cyclic GMP values were maximally elevated within 5 min and then quickly declined. By 60 min following hBNP treatment, plasma cyclic GMP levels were higher in the subcutaneous administration group than in the intravenous group (P < .005).

**Cardiovascular and renal effects in anesthetized rabbits.** Treatment with 30 μg/kg hBNP delivered by either subcutaneous or intravenous protocols resulted in a significant increase in the rates of urine flow and sodium excretion (fig. 4). When delivered by the intravenous route, most of the renal response to hBNP occurred within the first 20-min collection period. When delivered by the subcutaneous route, most of the renal response to hBNP occurred during the first two 20-min collection periods (P < .05). There was no change in renal function following treatment with saline.

Treatment with 30 μg/kg hBNP delivered by either subcutaneous or intravenous protocols resulted in a significant decrease in systolic and diastolic blood pressures (fig. 5). When compared with base-line values, intravenous and subcutaneous delivery of hBNP were associated with a peak decrease in systolic blood pressure of 18 ± 3 and 19 ± 3 mm Hg, respectively, and a peak decrease in diastolic blood pressure of 7 ± 1 and 7 ± 2 mm Hg, respectively. Following drug administration, systolic blood pressure remained significantly below base-line levels for 50 and 150 min in the intravenous and subcutaneous delivery protocols, respectively (P < .05).

**Discussion**

This is the first report demonstrating that hBNP can be efficiently delivered by a subcutaneous route of administration and elicit significant hemodynamic and renal responses. These data suggest that subcutaneous treatment might be an effective method for delivering the peptide in human clinical trials.

Previous studies have demonstrated that intravenous administration of hBNP to rabbits results in an elevation of plasma cyclic GMP, reduced systolic blood pressure, natriuresis and diuresis (Clemens et al., 1997). The time-related increase in plasma cyclic GMP is consistent with activation of the guanyl cyclase-A receptor. Reduced blood pressure following hBNP treatment of normotensive animals has been shown to result from reduced cardiac preload resulting in reduced cardiac output (Clavell et al., 1993). Natriuresis and diuresis following hBNP treatment is generally believed to result from increased glomerular filtration rate and reduced reabsorption of tubular sodium.

Subcutaneous administration of hBNP to normotensive rabbits resulted in reduced systolic blood pressure, similar in magnitude but prolonged in duration when compared to the effects of hBNP given intravenously. A greater reduction in systolic rather than diastolic blood pressure was seen with hBNP administered by both protocols, consistent with the preload effects of hBNP, which have been described in pre-
previous studies in dogs (Clavell et al., 1993). Subcutaneous
treatment with hBNP resulted in a significant diuresis and
natriuresis. While the overall magnitude of the renal effect of
hBNP was similar in the two delivery protocols, the effect
was more prolonged in the subcutaneous treatment group.

Significant circulating concentrations of immunoreactive-
hBNP were seen in animals given the peptide subcutane-
ously. Area under the plasma concentration-time curves of
hBNP delivered by the two protocols suggests that up to 60%
of the hBNP delivered by the subcutaneous route is found in
the plasma. This assumes that with the intravenous administra-
tion protocol, 100% of the hBNP was delivered to the
plasma compartment, all of the immunoreactive material
detected in the plasma is intact and/or biologically active and
metabolism of hBNP
via peptidases specific to the subcuta-
aneous pathway does not result in the formation of a hBNP
species with enhanced affinity for the antibody used in the
immunoassay. As the biological response to subcutaneous
hBNP was comparable in magnitude and more prolonged in
duration than intravenous hBNP, it is clear that significant
circulating levels of hBNP were achieved by the subcutane-
ous protocol.

Subcutaneous administration of hBNP resulted in plasma
consentations of hBNP-immunoreactive material 15, 30 and
60 min after treatment of 2.2 ± 1.0, 2.4 ± 0.6 and 1.7 ± 0.5
nM, respectively. As hBNP activates the rabbit GC-A recep-
tor with an ED$_{50}$ of 7.2 ± 1.9 nM (A. Protter, data not shown),
these circulating levels are biologically meaningful. While
plasma hBNP levels 60 min following subcutaneous delivery
remain significantly elevated, the plasma concentration at
this time after intravenous treatment is only 0.12 ± 0.03 nM.
The more rapid loss of circulating hBNP following intrave-
nous delivery compared with subcutaneous delivery is con-
sistent with the shorter duration of biological effects of hBNP
given by the former protocol.

The effectiveness of subcutaneous delivery of hBNP demon-
strated here in rabbits suggests that this mode of admin-
istration might be applied therapeutically in humans. Con-
flicting results of subcutaneous delivery of the structurally
related peptide, synthetic human ANP, have been reported
(Crozier et al., 1987; Osterode et al., 1995). Characterizing
ANP delivery to the circulation by area under the curve
analysis of immuno-reactive material, bioavailability esti-
mates of 3% (Crozier et al., 1987) and 22% (Osterode et al.,
1995) were reported. No significant renal or hemodynamic
effects were reported following ANP subcutaneous treatment
studies, although one report demonstrated that subcutane-
ous ANP induced a significant increase in plasma cyclic
GMP, an effect consistent with activation of the biological
receptor for ANP.

Bolus intravenous administration of hBNP (Hobbs et al.,
1996) to patients with congestive heart failure has demon-

![Fig. 4. Rates of urine flow (A) and urine sodium (B) in rabbits treated
with 30 μg/kg hBNP by intravenous (○, n = 10) or subcutaneous (△, n =
12) delivery protocols compared with 0.9% NaCl treated animals (□, n =
11). Drug was administered at t = 0. Data are mean ± S.E.M. * P < .05
and ** P < .01.](image)

![Fig. 5. Change from base line of systolic (A) and diastolic (B) blood
pressures in rabbits treated with 30 μg/kg hBNP by intravenous (○, n =
10) or subcutaneous (△, n = 12) delivery protocols compared with 0.9%
NaCl-treated animals (□, n = 11). Drug was administered at t = 0. Data
are mean ± S.E.M. * P < .05 and ** P < .01.](image)
strated beneficial hemodynamic effects, including decreased pulmonary capillary wedge pressure and increased cardiac index. Subcutaneous administration may increase the duration of hBNP’s beneficial effects thereby simplifying treatment protocols. In addition, a subcutaneous delivery method might allow testing for beneficial effects of long term hBNP treatment.

Acknowledgments
The authors thank Lisa Gregory and John Lewicki for critical review of the manuscript and Larry Carstensen for animal maintenance.

References

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