AlbuBNP, a Recombinant B-Type Natriuretic Peptide and Human Serum Albumin Fusion Hormone, as a Long-Term Therapy of Congestive Heart Failure

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Received May 16, 2004; accepted July 24, 2004

Purpose. B-type natriuretic peptide (BNP) has been in clinical use for the treatment of decompensated congestive heart failure. However, BNP has a very short half-life in circulation, which limits its application to acute CHF and requires continuous i.v. infusion. To provide superior pharmacological benefits of BNP to other stages of chronic congestive heart failure and to eliminate problems associated with drug delivery via continuous i.v. infusion, we have designed and evaluated AlbuBNP, a long-acting form of BNP by recombinant fusion to human serum albumin for use in chronic congestive heart failure, post-acute follow-up, and postmyocardial infarction.

Methods. Human BNP (1–32) was seamlessly fused to mature human serum albumin at N-terminus to create AlbuBNP. The bioactivities of AlbuBNP were evaluated by natriuretic peptide receptor-A mediated cGMP activation assay, hemodynamic responses, and plasma cGMP elevation. The pharmacokinetic properties were determined after single i.v. or s.c. bolus injection in C57/BL6 mice.

Results. AlbuBNP had approxiamtely the same maximal bioactivity as BNP to activate cGMP in the *in vitro* NPRA/cGMP assay. The EC50s were 28.4 ± 1.2 and 0.46 ± 1.1 nM for AlbuBNP and BNP, respectively. In spontaneously hypertensive rats, AlbuBNP lowered both systolic and diastolic blood pressure, having sustainable mean arterial pressure reduction for more than 2 days. Six nmol/kg AlbuBNP i.v. bolus in mice increased plasma cGMP level 5.6-fold over the baseline. The elimination half-life in mice was dramatically increased from 3 min for BNP to 12–19 h for AlbuBNP.

Conclusions. AlbuBNP is bioactive and has desired pharmacokinetic properties for long-term use. It has the potential to be further developed as a new therapeutic option for chronic, acute, and post-acute CHF to alleviate symptoms, improve clinical status, and slow the disease progression by sustained drug exposure via infrequent simple subcutaneous injections.

KEY WORDS: B-type natriuretic peptide; congestive heart failure; serum albumin fusion.

INTRODUCTION

Heart failure is the final pathologic outcome of a constellation of cardiovascular diseases that impair heart pumping function, with coronary artery disease and myocardial infarction as the most common underlying causes. The prevalence of CHF is close to 5 million in the United States and 20 million worldwide. Current therapies include ACE inhibitors, nitrates, dopamine, beta-blockers, inotropic agents, and diuretics. These drugs generally act on one aspect of pathophysiology of CHF, few of which reduce mortality rate and prolong lives.

B-type natriuretic peptide (BNP) has many advantages for CHF treatment (1–14). It is a natural hormone produced primarily in ventricle in response to cardiac overload. Hemodynamically, it is a balanced vasodilator that reduces both cardiac preload and after preload (15,16). From neurohormonal perspective, BNP reduces and counteracts the excessive renin/angiotensin, endothelin, and aldosterone that increase vascular resistance, cardiac load, and volume pressure, protecting the heart from compensatory stress and damage (17–22). The diuretic and natriuretic activities also help to decrease blood volume and relieve fluid congestion (23). The safety, efficacy, and therapeutic benefits to treat advanced acute heart failure have been demonstrated by the VMAC (6,24,25) and PRESEDENT clinical studies (26). BNP has been a prescription drug as Natrecor (nesiritide).

Due to its rapid clearance and the need for continuous intravenous infusion, current use of BNP is limited to decompensated acute heart failure in the hospital setting (27–29). The prospect of long-term application of BNP for earlier stages of chronic heart failure, post-acute follow-up, and postmyocardial infarction has its sound rationales. As heart failure is a progressive disease, it is necessary to establish continual hemodynamic profile favorable to heart failure condition and to maintain a neurohormonal balance to protect the heart from further deterioration. As heart failure is a debilitating disease, it is also important to ameliorate heart failure syndromes and to improve clinical status not only in emergency situations but also in everyday life. BNP has properties that are particularly valuable for long-term use. Unlike other heart drugs that are helpful in short term but harmful in long term, BNP is not inotropic, chronotropic, and proarrhythmic (3,16,30). Compared with nitrates that develop drug tolerance quickly, no tolerance to BNP has been observed (6,24–26). In fact, a heart transplantation candidate patient who received more than 5 months of continuous infusion responded to the nesiritide treatment well (25). In BNP transgenic mice, constitutive overexpression of BNP produced no harmful effects that are relevant to BNP use in patients (31). The excellent long-term safety profile is highly desirable for prolonged treatment. Moreover, BNP has been reported to limit myocardial infarction (32), slow the progression of cardiac hypertrophy and ventricular remodeling (33), and improve neovascularization of the ischemic lesion (34). These functions beneficial to heart failure condition are only meaningful when the BNP activities are sustained. The Follow-up Serial Infusion of Nesiritide (FUSION) clinical study has in fact demonstrated the efficacy and safety of in longer term post-acute heart failure management (35). When administered as weekly 6-h per day continuous i.v. infusions for 12 weeks on outpatients with advanced congestive heart failure, BNP resulted in statistically significant reductions in death and all-cause hospitalization compared with standard care. The FUSION group had higher survival rates, overall clinical status improvement, and, remarkably, reversal of New York Heart Association (NYHA) heart failure severity class.

We have investigated AlbuBNP, a BNP and human serum albumin recombinant fusion hormone, for sustained *in vivo* BNP activities. HSA is the most abundant natural carrier

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protein in plasma and has a half-life exceeding 19 days in human. BNP and HSA fusion could reduce proteolytic BNP cleavage by steric hindrance, NPR-C receptor mediated clearance, and renal filtration of BNP as small peptide (36–43). Our investigation showed that AlbuBNP is bioactive with dramatically improved pharmacokinetic and pharmacodynamic characteristics ideal for long-term heart failure medication.

MATERIALS AND MEHODS

Expression Constructs for Recombinant AlbuBNP

The expression construct for AlbuBNP production was pC4:SPCON.BNP1-32/HSA. The construct encodes secrecon (44) as the signal peptide, human BNP1-32 and mature HSA. Human BNP1-32 sequence 5′-AGCCCCAAGATGGTGCAAGGGTCTGGCTGCTTTGGGAGGAAGATGGACCGGATCAGCTCCTCCAGTGGCCTGGGCTGCAAAGTGCTGAGGCGGCAT-3′ was PCR amplified with primers 5′-GAGCGCGGATCCAAGCTTCCGCCATCATGTGGTGGCGCCTGTGGTGGCTG CTGCTGCTGCTGCTGCTGCTGTGGCCCATGGTGTGGGCCAGCCCCAAGCTGGTGCAAGG-3′ and 5′-AGTCCCATCGATGAGCAACCTCACTCTTGTGTGCATCATGCCGCCTCAGCACTTTGC-3′. The PCR product was digested with *Bam*HI and *Cla*I. The insert was cloned into pC4HSA vector (HGS proprietary mammalian expression vector) cut with *Bam*HI and *Cla*I.

To generate pC4:SPCON.BNP1-32(2x)/HSA where BNP moiety is duplicated, two DNA fragments were amplified by PCR: primers 5′-AGCCCCAAGATGGTGCAAGGGTCTGGCTGCTTTGGGAGGAAGATGGACCGGATCAGCTCCTCCAGTGGCCTGGGCTGCAAAGTGCTGAGGCGGCAT-3′ and 5′-CCTTGCACCATCTTGGGGCTATGCCGCCTCAGCACTTTGC-3′ for Fragment A, primers 5′-GCAAAGTGCTGAGGCGGCATAGCCCCAAGATGGTGCAAGG-3′ and 5′-AGTCCCATCGATGAGCAACCTCACTCTTGTGTGCATCATGCCGCCTCAGC ACTTTGC-3′ for Fragment B, both using BNP1-32 sequence as the template. The two fragments were purified by agarose gel electrophoresis and mixed in equal molar amount as the PCR template. The BNP(2x) insert was amplified by PCR using the same primer pairs for pC4:SPCON.BNP1-32/HSA for the plasmid construction as described above, digested with *Bam*HI and *Cla*I and cloned into pC4HSA vector pre-digested with *Bam*HI and *Cla*I. The expression constructs were verified by full length sequencing.

Recombinant Protein Production

293F cells were transfected with pC4:SPCON.BNP1-32/HSA plasmid DNA by Lipofectamine method (Invitrogen, Carlsbad, CA, USA). The transfected cells were grown in CHO5 serum-free medium. Two liters of supernatant were collected 3 days post-transfection.

The recombinant protein was captured by 5 ml Blue Sepharose CL-6B column (Amersham Biosciences, Piscataway, NJ, USA) and eluted by 2 M NaCl. The material was bound to HiPrep 16/10 Phenyl FF (high sub) column and eluted by 20 mM MES, pH 6.7. AlbuBNP was further purified by hydroxyapatite column chromatography in sodium phosphate buffer gradient (0–20 mS/cm in 200 ml) at pH 6.8. The final product was exchanged into PBS pH 7.2 by a HiPrep 26/10 desalting column (Amersham Biosciences).

In Vitro NPRA/cGMP Assay

The open reading frame of human natriuretic peptide receptor-A (NPRA) was constructed into pcDNA3.1 expression vector (Invitrogen). 293F cells were stably transfected with the plasmid DNA by Lipofectamine method and selected by 0.8 μg/ml G418. 293F/NPRA stable clones were screened for best response to human BNP.

cGMP activation by BNP was carried out in 293F/NPRA cells and measured by CatchPoint cyclic-GMP fluorescent assay kit (Molecular Devices, Sunnyvale, CA, USA). Briefly, 50,000 cells/well of 293F/NPRA cells cultured in a 96-well plate were washed into 80 μl prestimulation buffer (Krebs-Ringer Bicarbonate Buffer with 10 mM glucose, pH 7.4, 15 nM sodium bicarbonate, and 0.75 mM 3-isobutyl-1-methylxanthine). AlbuBNP or BNP in 40 μl prestimulation buffer were added to the cells at 37°C for 10 min. The cells were lysed with 40 μl Lysis Buffer for 10 min with shaking. The amounts of cGMP in the lysates were quantitated as per the manufacturer's instruction.

Blood Pressure Measurement

Three-month-old male spontaneously hypertensive rats (SHR) were purchased from Taconic (Germantown, NY, USA). All animal procedures were approved by the Institutional Animal Use Committee at Human Genome Sciences, Inc.

AlbuBNP or BNP were reconstituted in 0.3 cc PBS per rat. The drugs were delivered via tail vein injection. Systolic and diastolic blood pressures were recorded by cuff-tail method using XBP-1000 System (Kent Scientific, Torrington, CT, USA). For each blood pressure data point, 4~5 consecutive readings were taken and averaged. Mean arterial pressure (MAP) was calculated as 1/3 systolic pressure + 2/3 diastolic pressure. For dose-response determination, blood pressures were measured 20 h after AlbuBNP administration at doses of 0.5, 2, 6, and 18 nmol/kg.

Pharmacokinetics of AlbuBNP

Eleven- to 12-week-old male C57/BL6 mice (obtained from Ace Animals, Boyertown, PA, USA) weighed 25.1 ± 0.12 g at the time of the study. All animals were dosed at a volume of 10 ml/kg body weight. Predose animals were injected with PBS. The proteins were injected intravenously in the tail or subcutaneously in the mid-scapular region.

The study was performed on the following groups:

Group	Drug	Dose (mg/kg)	Route	n/time	Time (hours)
1	AlbuBNP	2.19	SC	3	0.5, 2, 6, 16, 24, 32, 48, 72, 96
2	AlbuBNP	2.19	IV	3	0.083, 2, 6, 16, 24, 32, 48, 72, 96
3	Vehicle	0	SC	3	predose
4	Vehicle	0	IV	3	predose

Blood samples were obtained at 0.5, 2, 6, 16, 24, 32, 48, 72, and 96 h after s.c. injection. Blood samples were collected at 5 min, 2, 6, 16, 24, 32, 48, 72, and 96 h after i.v. injection. Blood was sampled from the inferior vena cava, placed into an EDTA-coated microtainer, and stored on ice. The samples were centrifuged in a microcentrifuge at 14,000 rpm (16,000 × *g*) for 10 minutes at room temperature. The plasma was transferred into cluster tubes and stored at –80°C.

AlbuBNP concentrations in plasma samples were determined using BNP EIA Kit (Phoenix Pharmaceutical, Belmont, CA, USA). The standard curves were generated at the same time on the same plate with testing samples. The detection limit was 0.11 ng/mL for AlbuBNP. The assay detects human BNP and does not cross react to mouse BNP.

Pharmacokinetic analysis was conducted by noncompartmental methods (WinNonlin; version 4.1; Pharsight Corp., Mountain View, CA, USA). The mean plasma concentration at each time was used in the analysis. A linear up/log down trapezoidal method was used to calculate the AUC_{0-t}. Extrapolation to infinity $AUC_{0-\infty}$ was done by dividing the last observed concentration by the terminal elimination rate constant. Data were uniformly weighted for these analyses.

In Vivo Plasma cGMP Levels as Pharmacodynamic Biomarker of AlbuBNP Action

Eleven- to 12-week-old male C57/BL6 mice received a single bolus of BNP or AlbuBNP at 6 nmol/kg dose via tail vein. Plasma were prepared from the tail bleeds at 5, 10, 20, 40, and 80 min time points for BNP dosing group and at additional 640, 1440, 2880, and 5760 min for AlbuBNP group. Plasma samples from mice treated with PBS as the vehicle control were collected as the zero time points. cGMP levels were determined by CatchPoint cyclic-GMP fluorescent assay kit according to the manufacture's instruction.

Immunogenicity Detection

The anti-BNP antibody immunodetection assay was established by coating 96-well SigmaScreen Streptavidin Coated Plates (Sigma-Aldrich, St. Louis, MO, USA) with 20 pmol/well synthetic BNP with lysine-biotin as the C-terminal residue. Biotinylated BNP coated plate was washed with PBS three times. Five-microliter serum samples were diluted in 100 μl PBS, and the binding was carried out at room temperature for 1 h with shaking. After washing three times with PBS, 100 μl protein-G peroxidase conjugate (Rockland Immunochemicals, Gilbertsville, PA, USA) at 1:50,000 dilution in PBS was added and incubated for 1 h. After washing with PBS three times, the amounts of anti-BNP antibody were measured by 50 μl ECL Western blot solution (Amersham Biosciences, Piscataway, NJ, USA) and a microplate luminometer (Dynex Technologies, Chantilly, VA, USA).

Spontaneously hypertensive rats were injected with 0.5 nmol/kg or 6 nmol/kg AlbuBNP, ratBNP/RAS, or PBS every 3 days for 2 months. Blood samples were collected from the tails. Five-microliter serum samples were detected for BNP immunoreactive antibody in the anti-BNP antibody immunodetection assay as described above.

RESULTS

Generation of Recombinant AlbuBNP

AlbuBNP is a seamless fusion of human BNP (1–32) with the sequence of SPKMVQGSGCFGRKMDRISSSSGLGCKVLRRH to the N-terminus of mature human serum albumin (Fig. 1, upper panel). For secretion of the precursor protein from mammalian producer cells, signal peptides of human serum albumin (HSASP, MKWVTFISLLFLFSSAYSRGVFRR-), chemokine MPIF (MPIFSP, MKVSVAALSCLMLVTALGSQA-) and artificial secrecon (MWWRLWWLLLLLLLLWPMVWA-) (44) were compared. Recombinant proteins were expressed by transient transfection in 293F cells. The purified proteins were microsequenced to examine the integrity of the N-termini. AlbuBNPs precursors with HSASP and MPIFSP as the signal peptide had significant signal peptide miscleavage. Because residual amino acid residues left by the signal peptide miscleavage present potential source of immunogenicity, we have adopted secrecon for its accurate and clean signal peptide removal during protein secretion as well as the highest protein expression and yield.

The purified recombinant AlbuBNP concentration was typically 1~2 mg/ml with a yield approximately 20 mg per liter supernatant. The estimated purity was >90% by SDS-polyacrylamide gel electrophoresis, with a molecular weight of 70 kDa (Fig. 1, lower panel). The AlbuBNP protein had the correct N-terminal sequence of SPKM-. Compared with the calculated formula weight of 69919.43 Da., mass spectroscopy identified two peaks of molecular weights of 69879.81 and 70000.58 Da. The 120 Da delta mass between the peaks suggests an occurrence of cysteinylation. The AlbuBNP preparation had a low endotoxin level <0.1 EU/ml.

In an attempt to increase the bioactivities of the fusion protein, we duplicated BNP (1–32) moiety with the sequence of SPKMVQGSGCFGRKMDRISSSSGLGCKVLRRHSPKMVQGSGCFGRKMDRISSSSGLGCKVLRRH to create BNP(2x)/HSA (Fig. 1, upper panel). Without adding

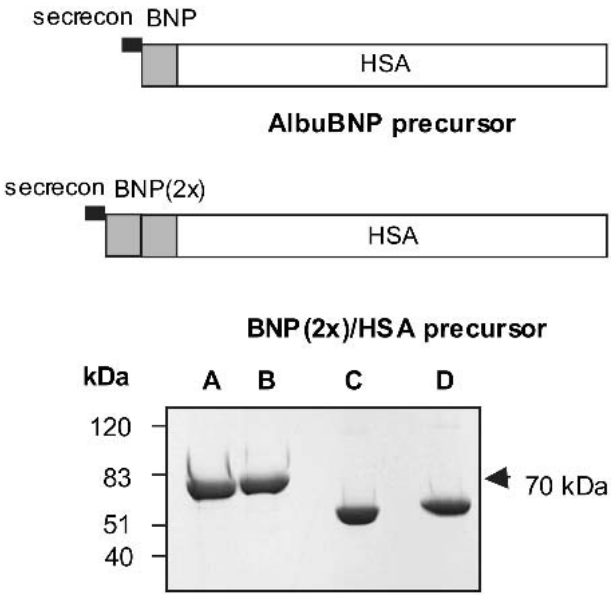

Fig. 1. Schematic diagram of AlbuBNP and BNP(2x)/HSA precursors. The signal peptide is adopted from the secrecon sequence (MWWRLWWLLLLLLLLWPMVWA). BNP, mature human BNP(1-32); BNP(2x), duplicated BNP(1-32); HSA, mature human serum albumin. Lower panel, SDS-polyacrylamide gel electrophoresis analysis of purified mature AlbuBNP (A and C) and BNP(2x)/HSA (B and D) proteins under reducing (A and B) and non-reducing (C and D) conditions.

a potentially immunogenic extragenous sequence as a linker, the BNP duplication could provide more space between the first BNP and HSA to reduce steric hindrance. Moreover, two copies of BNP may have additive effect. The protein expression, bioprocessing, bioactivities, and pharmacokinetics of BNP(2x)/HSA were also evaluated.

AlbuBNP Is Active in NPR-A–Mediated cGMP Signaling

Among natriuretic peptide receptors NPR-A, NPR-B and NPR-C, NPR-A is the signaling receptor for BNP, responsible for most of the biological effects. BNP bioactivity is mediated by NPR-A guanylyl cyclase domain that converts GTP to cGMP upon activation. We have engineered a stable NPR-A overexpressing 293F cell line that responds to BNP stimulation. The cGMP production in the cells was measured by competitive cGMP ELISA.

The dose-response relationship of AlbuBNP, BNP(2x) and BNP were determined by the NPRA/cGMP assay. The maximal activities of AlbuBNP and BNP(2x)/HSA approached that of BNP (Fig. 2). AlbuBNP, BNP(2x)/HSA and BNP had EC50 28.4 ± 1.2, 9.8 ± 1.1, and 0.46 ± 1.1 nM respectively.

Even though BNP(2x) had much higher activity than AlbuBNP as theorized, there was excessive cleavage between K13 and R14 residues in the BNP moieties of the BNP(2x)/HSA molecule during protein expression in 293F and CHO cells, most likely by chymotrypsin type proteolytic degradation. Further exploration of other expression systems for scalable BNP(2x)/HSA production is needed. Instead, AlbuBNP was chosen for further characterization.

Effects on Blood Pressure

BNP reduces blood pressure by direct vasodilation as well as by suppression of renin/angiotensin/endothelin/aldosterone systems. We tested *in vivo* activity of AlbuBNP to decrease arterial blood pressure in spontaneously hypertensive rats. Six nmol/kg AlbuBNP was delivered into spontaneously hypertensive rats via tail vein i.v. injection. The systolic and diastolic blood pressures were measured by cuff-tail method. Spontaneously hypertensive rats are genetically hypertensive with onset of high blood pressure after three months of age. Typical systolic pressure was 180–200 mmHg before AlbuBNP dosing. Single bolus of 6 nmol/kg AlbuBNP lowered both systolic and diastolic pressure, which accounted for more than 30 mmHg mean arterial pressure (MAP) reduction. The lowered blood pressure was steady and continued for a day and then gradually returned to the baseline over several days (Fig. 3A). The prolonged exposure to AlbuBNP did not cause continued blood pressure decline, as in the case of continuous BNP infusion in other studies where hymodynamic responses are dose-dependent but not time-dependent. Due to its instantaneous clearance, single BNP bolus at the same dose produced only very transient MAP decrease about ~15 mmHg.

The AlbuBNP and blood pressure dose-response was established in SHR (Fig. 3B). AlbuBNP 0.5 nmol/kg had an average of 7 mmHg MAP reduction. High dose at 18 nmol/kg lowered the blood pressure only slightly more than 6 nmol/kg, suggesting a plateau the hormone could reach.

Plasma cGMP Levels

The intracellular cGMP activation by BNP results in its release from the cell to circulation. The plasma cGMP level correlates with BNP induced cardiovascular and renal physiology. Plasma cGMP has been used as a biomarker for *in vivo* BNP action (45–47).

Mice received single i.v. bolus of 6 nmol/kg BNP or AlbuBNP had peak plasma cGMP levels over the baseline 3.9- or 5.6-fold respectively (Fig. 4). The one-phase exponen-

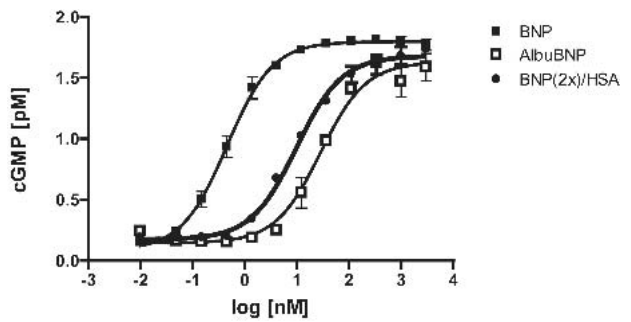

Fig. 2. Dose-response relationship of AlbuBNP, BNP(2x)/HSA, and BNP in NPRA/cGMP assay. The maximal cGMP activation activities of AlbuBNP, BNP(2x)/HSA, and BNP were 1.63 ± 0.016, 1.68 ± 0.021, and 1.80 ± 0.016 pM. The EC50 values of AlbuBNP, BNP(2x)/HSA, and BNP were 28.4 ± 1.2, 9.8 ± 1.1, and 0.46 ± 1.1 nM. The semilog curves were fitted by sigmoidal dose-response nonlinear regression.

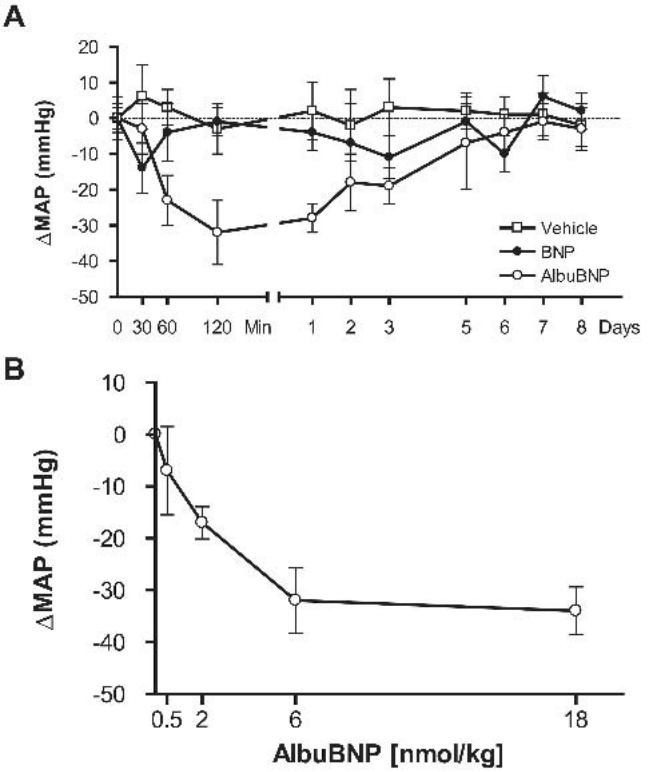

Fig. 3. (A) Mean arterial pressure reduction (ΔMAP) by 6 nmol/kg AlbuBNP or BNP in spontaneously hypertensive rats (n = 4). The vehicle control was PBS with 6 nmol/kg recombinant HSA. (B) Dose-response of ΔMAP 20 h post single bolus i.v. injection of AlbuBNP in spontaneously hypertensive rats (n = 4). Blood pressure was measured by cuff-tail method.

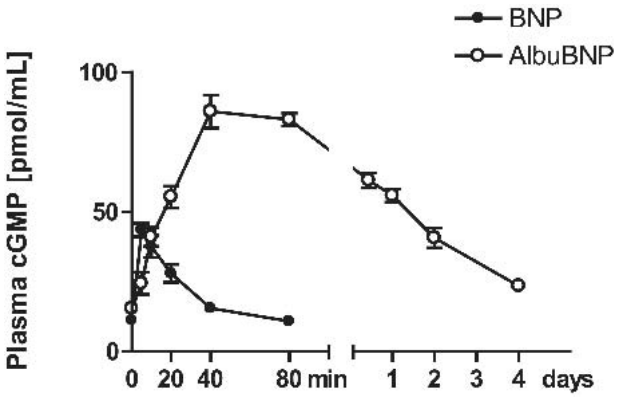

Fig. 4. Plasma cGMP levels in mice (n = 4) after single bolus i.v. injection of 6 nmol/kg AlbuBNP or BNP. Error bars are SEM.

tial decay half-life of cGMP was 16 min (10 to 42 min, 95%CI) by BNP or 1538 min (1017 to 3153 min, 95%CI) by AlbuBNP administration.

Pharmacokinetic Properties

The mean baseline concentration of AlbuBNP in plasma as detected in the pre-dose samples was approximately 0.081–0.095 μg/ml, which were subtracted from all subsequent concentration measurements for the graphic presentation (Fig. 5) and PK analysis. The mean plasma (±SD) concentrations following a single i.v. or s.c. injection of AlbuBNP, BNP(2x)/HSA, and BNP are shown in Fig. 5. AlbuBNP had terminal elimilation half-lives of 11.2 or 19.3 h after i.v. or s.c injection. BNP(2x)/HSA had a shorter half-life of 4.6 h than AlbuBNP. The half-life of human BNP in mice was 3.1 min, which is extremely short as in the case of rat BNP in rats (1.2 min) (29) and human BNP in human (18 min) (27). Results of non compartmental analysis of AlbuBNP are shown in Table I. Five points at the terminal phase of the i.v. profile and four points at the terminal phase of the s.c. profile are selected for the terminal half-life calculation. The resulting AUC during this terminal phase is approximately 10% of the total AUC for the i.v. and s.c. profiles, respectively. This is compared to only 2% and 4% of the total AUC for the i.v. and s.c. profile, respectively, when the last three points are selected for the terminal half-life calculation. As seen in Fig. 5, the s.c. profile consists of a first-order absorption followed by a possible biphasic pattern, with an inflation point seen at 48 h The biphasic profile is less obvious for the i.v. group with the inflation points occurring between the 48 h and 72 h time points. Future studies including an additional sampling point beyond 96 h will help confirm this biphasic nature observed in the current study. For the i.v. group, the profile seen during the first 6 h also suggests the potential for a capacity-limited mechanism with large dose (Michaelis-Menten kinetics). A future study using a lower dose level will help confirm this observation.

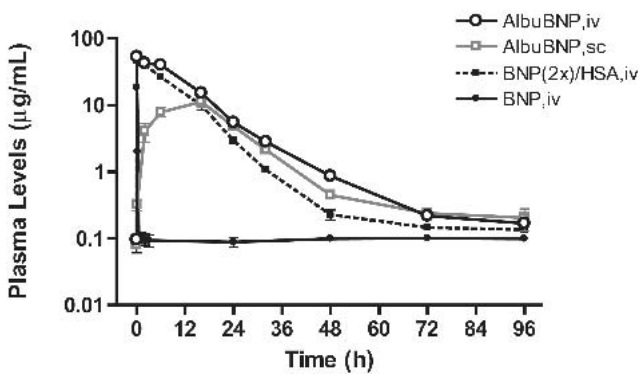

Fig. 5. Time course of plasma AlbuBNP, BNP(2x)/HSA, and BNP levels in C57/BL6 mice (n = 4) after i.v. or s.c. injection at an initial dose of 32 nmol/kg.

Table I. Pharmacokinetics Following a Single Dose of 2.19 mg/kg AlbuBNP in Mice

	Unit	i.v.	s.c.
t_{max}	h	NA	16
C_{max}	μg/ml	NA	11.2
$t_{1/2,term}$	h	11.2	19.3
$AUC_{0-\infty}$	(h · μg/ml)/(mg/kg)	658.9	227.9
V_{ss}	ml/kg	37	NA
V_z or V_z/F	ml/kg	53.5	268
CL or CL/F	ml/h/kg	3.3	9.6
MRT	h	11.2	19.8
Bioavailability	%		34.6

C_{max}, peak plasma concentration of the drug; t_{max}, time of maximum plasma concentration; $AUC_{0-\infty}$, area under the plasma drug concentration-time curve from time 0 to infinite time; $t_{1/2,term}$, terminal elimination phase half-life; CL, clearance after i.v. dosing; CL/F, apparent clearance after s.c. dosing; V_{ss}, volume of distribution at steady-state after i.v. dosing; V_z: volume of distribution during the terminal phase after i.v. dosing; V_z/F, volume of distribution during the terminal phase after s.c. dosing; NA, not applicable.

DISCUSSION

With better understanding of heart failure pathophysiology, treatment of congestive heart failure has made significant progress over the past several decades. In particular, the use of ACE inhibitors and beta-blockers to improve survival rate illustrates the importance of reducing cardiac load for chronic heart failure patients. However, heart failure remains a deadly and debilitating disease. A more effective and convenient option is needed. BNP has proven superior for advanced acute heart failure. The pharmacological properties of BNP, if sustained, are well suited for chronic heart failure at all stages. In fact, experimental long-term i.v. infusion of BNP has been shown not only to slow the disease progression but also reverse NYHA severity classes. However, because of its short half-life, the need for long-term continuous i.v. infusion, and the high cost, BNP is not practical for chronic heart failure.

As reported here, AlbuBNP has many characteristics to warrant further development as a long-term therapy for heart failure: 1) The elimination half-life of AlbuBNP in mice is 19 h, which is a dramatic increase from 3 min for BNP. The dosing regimen is expected to be intermittent injections at a minimal interval greater than several days to provide sustained cardiac protection. 2) AlbuBNP can be delivered by simple s.c. injection instead of continuous i.v. infusion for 6 h per day as with nesiritide (the FUSION protocol). 3) AlbuBNP is not excreted, likely less susceptible to NPR-C or neutral endopeptidase mediated clearance. Therefore, long-

acting AlbuBNP, as demonstrated by its PK/PD, would require less amount of the drug and thus the costs than BNP. Compared with ACE inhibitors, AlbuBNP is more effective in reducing preload without causing symptomatic hypotension. Based on BNP clinical experience, AlbuBNP would not have adverse effects such as dry cough, headache, dizziness, skin rash, kidney problems, and swelling like ACE inhibitors. As patients with low renin levels are refractory to ACE inhibitor treatment, AlbuBNP will continue to act on cardiovascular system independent of angiotensin/renin system (48). In addition, the combination of other functions of BNP in diuresis, natriuresis, suppression of angiotensin/endothelin/aldosterone, infarction protection, ventricular remodeling prevention offers AlbuBNP as overall a much better long-term management.

BNP is a balanced vasodilator that reduces both arterial and venous pressures, with venous effect more dominant. For congestive heart failure, the dose finding will need to be conducted in human subjects. Although arterial pressure reduction is desirable to decrease cardiac afterload and vascular resistance as demonstrated by the clinical benefits of ACE inhibitors, the optimal AlbuBNP dose should reduce cardiac preload without causing symptomatic hypotension. The blood pressure reduction by AlbuBNP has a plateau in the dose-response curve (Fig. 3B), which may be a result of natural hemodynamic compensation to hypotension. From a clinical perspective, this phenomenon may limit the risk of accidental overdose that could lead to uncontrolled hypotension.

One issue regarding AlbuBNP as an injectable drug is its potential immunogenicity, as the fusion of BNP and human serum albumin may create an antigenic epitope at the junction site. We have tested a rat version of AlbuBNP which a rat BNP(1–45) is seamlessly fused to the N-terminus of rat serum albumin. In rats injected with rat AlbuBNP at putative clinical dose (0.5 nmol/kg) or above the clinical dose (6 nmol/kg) every 3 days for 2 months, no anti-rat BNP immunoreactivity was detected, perhaps due to the low antigenicity score of BNP (Welling Prediction) (49). Less informatively, human AlbuBNP in rats produced low but detectable level of anti-human BNP antibody. The incidence of either non-neutralizing or neutralizing autoantibody against BNP by human AlbuBNP in human can only be investigated in human subject studies. At any rate, even if AlbuBNP induces some level of immunogenicity, there would be no serious health consequence as BNP has natural redundancy of ANP whose sequence is sufficiently divergent from BNP. In fact, BNP genetic knockout mice are generally healthy. BNP null knockout mice are viable throughout adulthood. There are no differences in gross heart anatomy, heart-to body weight ratio, ventricular hypertrophy markers Acta1 and Atp2a2, hematocrit, serum Na, K concentrations, aldosterone levels, plasma and urinary cGMP levels, or histology of any parts of the body among homozygous, heterozygous, and wild-type genotypes. All major BNP physiology including hemodynamic activity, natriuretic and diuretic activities, and electrolyte hemeostasis are preserved. No difference in cardiac hypertrophy under ventricular pressure overload induced by aortic constriction was found, nor significant blood pressure difference under high salt diet condition (50). Thus, AlbuBNP has wide safety margin against any potential immunogenicity. The concern of immunogenicity to reduce the efficacy of AlbuBNP is minimal as current medications, with the exception of nesiritide, act independently of BNP and continue to be available as fallback options. As heart failure is a life-threatening condition, the benefit-to-risk ratio warrants clinical development of AlbuBNP in human subjects in the treatment of heart failure.

ACKNOWLEDGMENTS

We thank Tatiana Tsareva, Jingli Cong, Renee Miceli, and Darryl Sampey for technical assistance, Dr. Qinghai Zhao and Dr. Mallika Singh for protein production, and Dr. Paul Moore and Dr. Indra Sanyal for support.

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