A Human Study to Evaluate Safety, Tolerability, and Cyclic GMP Activating Properties of Cenderitide in Subjects With Stable Chronic Heart Failure

Rika Kawakami[1], Candace Y.W. Lee[1], Christopher Scott[2], Kent R. Bailey[2], John A. Schirger[1], Horng H. Chen[1], Sherry L. Benike[1], Valentina Cannone[1,3], Fernando L. Martin[1], S. Jeson Sangaralingham[1], Tomoko Ichiki[1] and John C. Burnett Jr.[1]

Cenderitide is a novel designer natriuretic peptide (NP) composed of C-type natriuretic peptide (CNP) fused to the C-terminus of *Dendroaspis* natriuretic peptide (DNP). Cenderitide was engineered to coactivate the two NP receptors, particulate guanylyl cyclase (pGC)-A and -B. The rationale for its design was to achieve the renal-enhancing and antifibrotic properties of dual receptor activation, but without clinically significant hypotension. Here we report the first clinical trial on the safety, tolerability, and cyclic guanosine monophosphate (cGMP) activating properties of Cenderitide in subjects with stable heart failure (HF). Four-hour infusion of Cenderitide was safe, well-tolerated, and significantly increased plasma cGMP levels and urinary cGMP excretion without adverse effects with no change in blood pressure. Thus, Cenderitide has a favorable safety profile and expected pharmacological effects in stable human HF. Our results support further investigations of Cenderitide in HF as a potential future cGMP-enhancing therapeutic strategy.

Study Highlights

WHAT IS THE CURRENT KNOWLEDGE ON THE TOPIC?

☑ NPs possess cardiorenal protective and cGMP activating properties linked to pGC receptors. Cenderitide is a novel designer NP that is the only NP to coactivate the two known NP receptors (pGC-A/pGC-B). Cenderitide elicits in normal subjects natriuretic and diuretic effects, with preservation of GFR and with less hypotension than nesiritide and is less susceptible to NEP degradation.

WHAT QUESTION DID THIS STUDY ADDRESS?

☑ We defined for the first time the safety, tolerability, and cGMP activation of Cenderitide in humans with stable chronic HF.

WHAT THIS STUDY ADDS TO OUR KNOWLEDGE

☑ We established that Cenderitide in stable HF patients is well tolerated with a safety profile in line with the expected pharmacological effect including cGMP activation and without excessive hypotension.

HOW THIS MIGHT CHANGE CLINICAL PHARMACOLOGY OR TRANSLATIONAL SCIENCE

☑ Our findings provide for the first time in human HF new insight into the tolerability and safety of the designer NP Cenderitide, which coactivates both pGC-A and pGC-B, laying the foundation for larger clinical trials in HF.

Advances in peptide engineering have resulted in innovative peptide therapeutics in the treatment of human diseases such as diabetes with glucagon-like peptide 1 receptor stimulators, HIV to target critical molecular pathways with novel cell penetrating peptides, and more recently in heart failure (HF) with the peptide seralaxin.[1–4] Goals of peptide engineering include enhanced receptor activation, cotargeting of more than one peptide system, and increased stability of a peptide-to-enzymatic degradation.

Cenderitide (CD-NP) represents a novel designer natriuretic peptide (NP) which was engineered to coactivate the two particulate guanylyl cyclase (pGC) NP receptors pGC-A and pGC-B.[5,6] Such a peptide does not exist in nature, as the endogenous cardiac NPs (ANP and BNP) are ligands for pGC-A,[7,8] while the endothelial derived CNP activates pGC-B.[9,10] To achieve dual pGC-A and pGC-B activation, Cenderitide was designed by fusing the 22 amino acids of human CNP with the 15-amino acid C-terminus of DNP (**Figure 1**).[5,11,12] Importantly, activation of both receptors results in the generation of the second-messenger cyclic guanosine monophosphate (cGMP).[11] CNP has more potent antifibrotic properties than ANP and BNP, while also having less hypotension but lacks renal actions. Of note, CNP is the most susceptible NP to neprilysin (NEP) degradation, which

[1]Cardiorenal Research Laboratory, Department of Cardiovascular Medicine, Mayo Clinic, Rochester, Minnesota, USA; [2]Department of Health Science Research, Mayo Clinic, Rochester, Minnesota, USA; [3]Department of Clinical and Experimental Medicine, University of Parma Medical School, Parma, Italy. Correspondence: John C. Burnett, Jr. (burnett.john@mayo.edu)

Received 18 September 2017; accepted 28 November 2017; advance online publication 11 January 2018. doi:10.1002/cpt.974

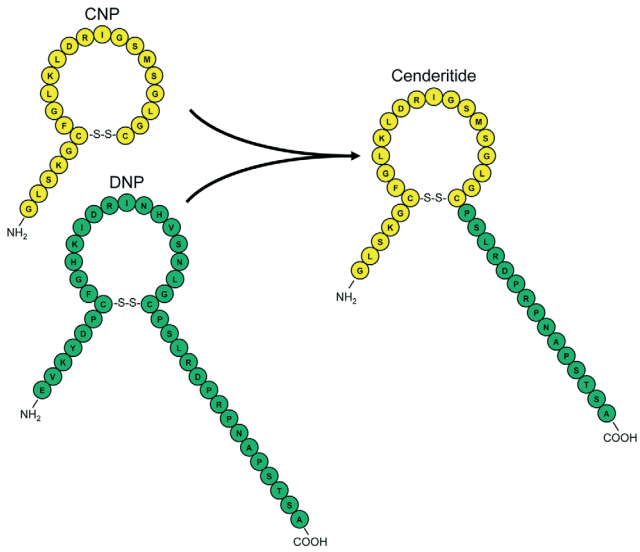

Figure 1 Structures and amino acid sequences of C-type natriuretic peptide (CNP), *Dendroaspis* natriuretic peptide (DNP), and Cenderitide (CD-NP).

has limited its clinical development.[13] Importantly, DNP has potent natriuretic and diuretic properties and is a robust activator of pGC-A.[14,15] Thus, a major goal in the engineering of Cenderitide was to create a novel designer NP that would cotarget pGC-A and pGC-B with less hypotension than pGC-A activators and which possessed antifibrotic properties like CNP but had the beneficial renal properties of pGC-A activation like DNP. Further, Cenderitide's design rendered it more resistant to NEP degradation than native NPs.[16]

Previous studies have reported that Cenderitide activates cGMP in human cardiac fibroblasts (CFs) and inhibits CF proliferation induced by profibrotic factors.[5] Further, Ichiki *et al.* reported that Cenderitide suppressed Collagen I gene expression activated by transforming growth factor beta (TGF-beta) and that these fibro-suppressing actions were greater than either BNP or CNP.[17] Further, in a rat model of cardiac fibrosis and impaired diastolic function, a 2-week continuous infusion of Cenderitide suppressed cardiac fibrosis and preserved myocardial diastolic function.[18] In normal canines, Cenderitide was diuretic, natriuretic, and increased the glomerular filtration rate (GFR) with less hypotension than BNP.[5]

To advance the clinical development of Cenderitide, Lee *et al.* administered Cenderitide to normal human volunteers in a first-in-human study to demonstrate the safety and tolerability of a 4-h intravenous infusion compared to placebo.[19] Cenderitide was well tolerated, without adverse actions and activated cGMP consistent with NP receptor activation. There was minimal blood pressure (BP) reduction, also consistent with the modest BP-lowering actions of CNP in humans together with natriuresis and diuresis, while GFR was preserved. Based on the safety and tolerability of Cenderitide in healthy subjects and its important cardiorenal profile, Cenderitide may have therapeutic potential for the treatment of HF, mediating cardiorenal protection.

The current study was designed as a prospective, randomized, placebo-controlled trial to determine for the first time the overall safety and tolerability of Cenderitide as well as the ability of Cenderitide to activate plasma and urinary cGMP in patients with stable chronic HF. We tested the hypothesis that Cenderitide can be safely administered to HF subjects and that the activation of cGMP observed in preclinical studies and in normal subjects can be translated to HF.

RESULTS

Demographics and baseline data

A total of 18 stable HF patients participated in this study. Of them, 12 were randomized to Cenderitide and 6 to placebo. **Table 1** summarizes the clinical characteristics of the study population.

Safety

The safety stopping criteria consisted of the following: 1) decrease in systemic systolic blood pressure from baseline by ≥30 mmHg, systolic BP <80 mmHg, or hypotension is associated with symptoms or that required treatment; 2) development of second- or third-degree atrioventricular (AV) block, interventricular conduction defect, corrected QT (QTc) interval >500 msec, or increase in the QTc interval by >30 msec during therapy, ventricular

Table 1 Baseline characteristics

	All (n = 18)	Placebo (n = 6)	Cenderitide (n = 12)	P- value
Age, years	63.2 ± 14.0	60.5 ± 15.1	64.5 ± 13.9	0.584
Male, n (%)	16 (88.9)	6 (100)	10 (83.3)	0.187
BMI, kg/m^2	29.9 ± 3.4	30.2 ± 3.7	29.7 ± 3.4	0.760
LVEF, %	28.5 ± 10.7	29.5 ± 14.2	28.0 ± 9.2	0.789
SBP, mmHg	108.5 ± 15.2	108.5 ± 15.7	108.5 ± 15.6	1.000
DBP, mmHg	63.4 ± 9.4	66.3 ± 7.9	61.9 ± 10.1	0.365
HR, beats/min	65.3 ± 11.5	63.5 ± 12.9	66.2 ± 11.2	0.656
SCr, mg/dl	1.10 ± 0.25	1.10 ± 0.17	1.10 ± 0.30	1.000

Data displayed as mean ± standard deviation, median (interquartile range), or n (%). BMI, body mass index; LVEF, left ventricular ejection fraction; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; SCr, serum creatinine.

Table 2 Treatment-emergent adverse events during the study

	Placebo, *n* = 6	Cenderitide, *n* = 12
Adverse effects		
Hypotension, *n* (%)		
Symptomatic hypotension	0 (0)	0 (0)
Symptomatic hypotension with SBP <80 mmHg	0 (0)	0 (0)
Decrease from baseline in SBP (≥30 mmHg)	0 (0)	0 (0)
Arrhythmia, *n* (%)		
Second- or third-degree AV block	0 (0)	0 (0)
Ventricular tachycardia > 5 beats	0 (0)	0 (0)
Ventricular fibrillation or asystole	0 (0)	0 (0)
Flushing, *n* (%)	0 (0)	0 (0)
Dizziness, *n* (%)	0 (0)	0 (0)
Tachycardia, *n* (%)	0 (0)	0 (0)
Paresthesia, *n* (%)	0 (0)	0 (0)
Dyspnea, *n* (%)	0 (0)	0 (0)

Data are presented as *n*. SBP, systolic blood pressure; AV, atrioventricular.

tachycardia >5 beats, ventricular fibrillation, or asystole in any subject.

All 18 patients completed the treatment period, and no patients met the prospectively defined stopping criteria. During Cenderitide infusion of 20 ng/kg/min, no significant changes in electrocardiographic findings were observed, nor did drug-related, clinically relevant changes in safety laboratory parameters occur. There were no adverse effects (**Table 2**).

Effects of Cenderitide

Neurohumoral parameters. Compared with Placebo, Cenderitide significantly increased plasma CNP levels as a measure of Cenderitide immunoreactivity (**Figure 2**). At baseline, plasma cGMP levels and urinary cGMP excretion were not significantly different between the two groups. The mean plasma cGMP levels and the mean urinary cGMP excretion were 3.9 ± 0.7 (mean ± standard error of the mean (SEM); range 1.1–12.3) pmol/mL and 905.5 ± 222.5 (mean ± SEM; range 268.3–3,540.1) pmol/min, respectively. During the 4-h infusion period, plasma cGMP levels and urinary cGMP excretion did not change in the placebo group, but after 4 h, plasma cGMP levels and urinary cGMP excretion of the Cenderitide groups were significantly increased from baseline (mean ± SEM, from 3.9 ± 0.9 to 8.8 ± 1.3 pmol/ml, $P < 0.01$ and 845.4 ± 279.7 to 2,176.5 ± 1,002.5 pmol/min, $P < 0.01$, respectively) (**Figure 2**). Also, plasma cGMP values of the Cenderitide group were significantly higher than those of the placebo group after 4 h of infusion (placebo 3.7 ± 1.3 to 3.6 ± 1.0 pmol/ml; Cenderitide 3.9 ± 0.9 to 8.8 ± 1.3 pmol/ml, $P < 0.05$). There were no changes in angiotensin II or aldosterone in either group.

Hemodynamic actions. The baseline hemodynamic variables including systolic blood pressure (SBP), diastolic blood pressure (DBP), and mean arterial pressure (MAP) and heart rate (HR) are illustrated in **Figure 3**. During 4 h of drug infusion, hemodynamic parameters remained unchanged in both groups.

Renal actions. Urine flow and urinary sodium excretion did not significantly differ between the placebo and Cenderitide groups after 4 h of study drug infusion. There was also no significant difference in GFR between the placebo and Cenderitide groups ($P = 0.165$, **Figure 4a,b**). Across the entire population of subjects receiving Cenderitide, there was no statistical change in GFR. However, in the Cenderitide subjects with low-baseline GFR equal or below 65 ml/min/1.73 m^2, mean GFR improved relative to baseline at 4 h (**Figure 4c**). The mean change from baseline in GFR was −7.1 ± 6.6 ml/min/1.73 m^2 in placebo, −15.6 ± 9.0 ml/min/1.73 m^2 in Cenderitide with high-baseline GFR (>65 ml/min/1.73 m^2), and +15.1 ± 4.5 ml/min/1.73 m^2 in Cenderitide with low-baseline GFR. Thus, the absolute increase in GFR during 4 h were significantly higher in Cenderitide patients with low-baseline GFR compared with placebo patients and Cenderitide patients with high-baseline GFR.

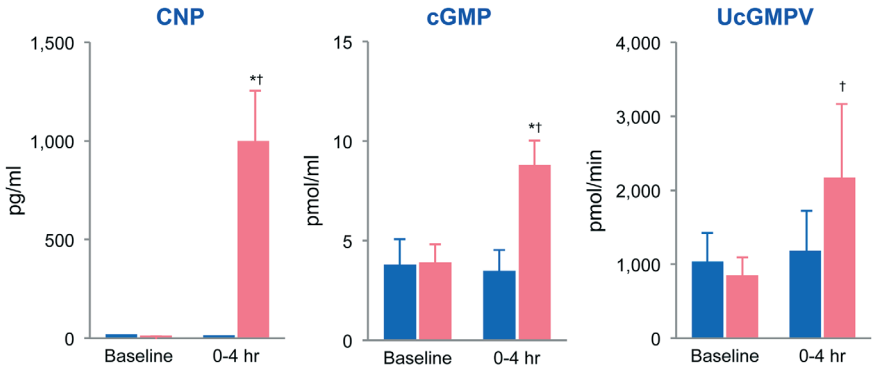

Figure 2 Effect of Cenderitide on plasma CNP, plasma cGMP, and urinary cGMP excretion (UcGMPV). Values are mean ± SEM. *$P < 0.05$ vs. placebo, †$P < 0.01$ vs. baseline.

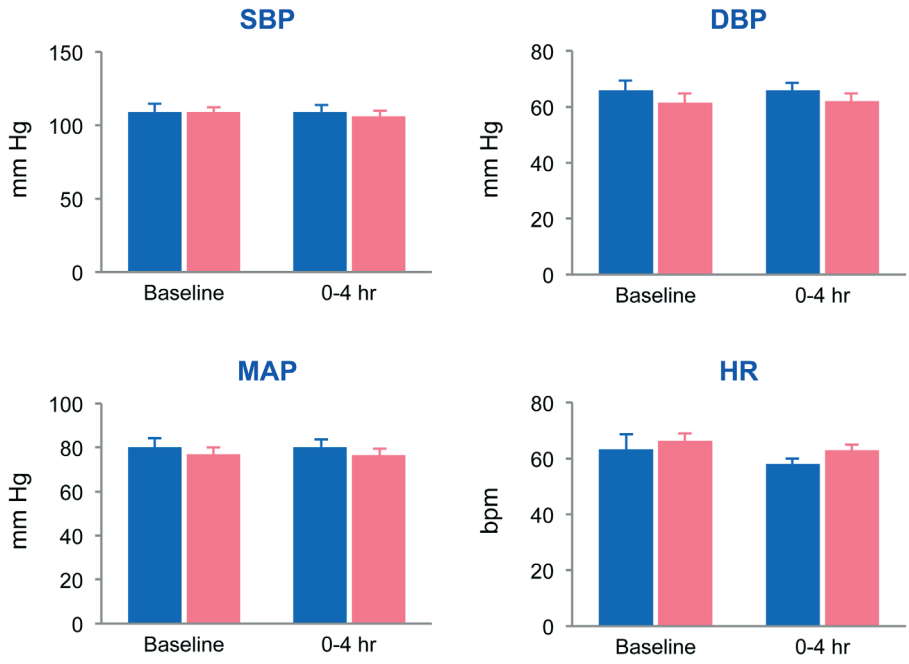

Figure 3 Effect of Cenderitide on systolic (SBP), diastolic (DBP), and mean arterial (MAP) pressures (mmHg). Values are mean ± SEM.

DISCUSSION

This clinical study investigated for the first time the safety, tolerability, pharmacodynamics, and cGMP activating properties of Cenderitide in patients with stable chronic HF. The study showed that a 4-h infusion of Cenderitide was safe, well tolerated with increases in plasma cGMP, and urinary cGMP excretion in the absence of hypotension. Importantly, GFR was preserved and was increased in the presence of a reduced GFR.

Nearly all etiologies of heart disease involve pathological myocardial remodeling characterized by excessive deposition of extracellular matrix proteins by CFs, which reduces tissue compliance and not only accelerates the progression to HF but also leads to poor prognosis in HF patients.[20,21] Indeed, left ventricular (LV) myocardium in both endstage HF and in LV assist device (LVAD) patients is characterized by the presence of fibrosis.[17] Thus, pathological fibrosis has emerged as an important target for pharmacological intervention in HF, yet approved drugs for this specific pathology are lacking. Cenderitide, as a dual pGC-A/pGC-B activator that may potentially optimize antifibrotic properties of the NPs/cGMP pathway, may represent a significant advance in targeting cardiac fibrosis.[5,17,22,23] Supporting the potential clinical target of fibrosis in HF is our demonstration of significant increases in plasma cGMP in the current study.

Impaired renal function characterized by a reduction in GFR and congestion secondary to sodium and water retention are more predictive of poor HF outcomes than LV ejection fraction (LVEF).[24,25] In normal canines, intravenous infusion of Cenderitide increased GFR and in freshly isolated canine glomeruli increased cGMP generation.[5,11] Cenderitide was also natriuretic and diuretic in normal dogs, with less reduction in BP compared

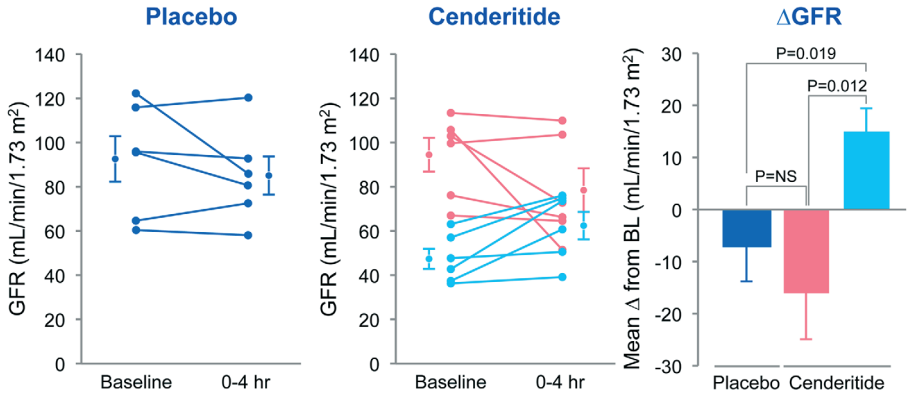

Figure 4 Individual GFR responses in placebo groups (**a**) and Cenderitide groups (**b**). Comparison of changes from baseline in GFR at 4 h between placebo, Cenderitide with baseline GFR ≥65 ml/min/1.73m^2, and Cenderitide with baseline GFR <65 ml/min/1.73m^2 groups (**c**). *P* value in box is from ANOVA for comparison of groups. Data are mean (SEM). NS, not significant.

to nesiritide.[5] In normal human volunteers, Cenderitide increased sodium and water excretion, preserved GFR, and augmented plasma and urinary cGMP excretion with a minimal reduction in BP.[19] In the current study in stable HF subjects, intravenous infusion of Cenderitide preserved GFR with an increase in plasma cGMP and urinary excretion of cGMP, with no change in blood pressure or sodium excretion. This reduced BP and natriuretic response may be due to reduced pGC-A and/or pGC-B receptor activity. Thus, in future studies a dose-ranging study of longer duration is warranted to address this issue. Alternatively, the lack of natriuresis and diuresis may be related to the short duration of infusion or the holding of diuretics on the day of the study.

In this small study we did note that GFR increased in subjects with GFR below 65 ml/min/1.73m^2 but this needs confirmation in a larger trial. These renal actions especially related to GFR are consistent with pGC-A action, as supported by previous studies in isolated glomeruli.[11] Of note, compared to placebo there was no natriuresis or diuresis.

Our major objective was to establish that Cenderitide was safe and well tolerated. In the current study there were no adverse effects observed. Specifically, there was no hypotension, tachycardia, dizziness, or other adverse effects.

It should be noted that Entresto represents a newly approved drug for HF that antagonizes the Angiotensin Type 1 receptor and inhibits neprilysin that is the enzyme especially abundant in the kidney that degrades the natriuretic peptides.[26] We previously reported a synergism in experimental HF with the acute coadministration of omapatrilat that inhibits both angiotensin converting enzyme and NEP in augmenting renal function.[27] Such studies provide a rationale for coadministration of Entresto and Cenderitide and warrant exploratory studies. To optimize such a small molecule and peptide cotherapy would require a chronic delivery strategy for Cenderitide. We would advance the strategy of subcutaneous delivery (s.q.) as is used for insulin. Such a delivery strategy is supported by our success with chronic delivery of BNP in patients with HF.[28,29] Finally, based on what we have learned in studies of Cenderitide in normal humans, in which cGMP was activated with natriuresis and in cultured human cardiac fibroblasts in which Cenderitide suppressed collagen gene expression greater than either BNP or CNP, one could make an informed assessment that a definitive trial in humans with HF to show renal enhancing and antifibrotic actions would be of 3-month duration, with s.q. Cenderitide at two doses, and be randomized, blinded, and placebo-controlled.[17,19]

Our study has limitations. It was a small, early-phase study and larger studies with more HF patients will be required to confirm the current findings. This would include a dose-ranging design to find optimal dosing strategies. Our study also was of short duration of Cenderitide as we assessed safety of 4 h of continuous intravenous infusion of 20 ng/kg/min that was a dose similar to the first-in-human study in normal volunteers, so longer-duration infusion studies are needed. Further studies also should more carefully assess interaction with diuretics.

In conclusion, this short-term Cenderitide study performed for the first time in human HF establishes that treatment with the novel designer NP Cenderitide that cotargets both pGC-A and pGC-B activates the cGMP pathway without changes in BP and HR in stable chronic HF patients. Importantly, Cenderitide was well tolerated, with a safety profile without adverse effects. We

Table 3 Key exclusion criteria

1.	History of allergy or other adverse reactions to exogenous natriuretic peptides (Cenderitide (CD-NP) or its components, nesiritide, other natriuretic peptides, or related compounds).
2.	Women who are pregnant, or breastfeeding.
3.	Nesiritide within 7 days prior to entry into the study.
4.	Any investigational drug or device within 30 days prior to entry into the study.
5.	Clinically unstable patients (e.g., systolic blood pressure <90 mmHg, ongoing requirement for vasopressors or mechanical circulatory support, or mechanical ventilation).
6.	Recent hospitalization for decompensated HF or recent defibrillation for cardiac resuscitation within 30 days prior to randomization.
7.	History of organ transplantation, on a waiting list for organ transplantation, or ongoing requirement for long-term vasoactive support.
8.	Patients with guarded prognosis who are unlikely to derive meaningful benefit from Cenderitide.
9.	Use of sulfonamides, nonsteroidal antiinflammatory drugs, probenecid, or other drugs that are known to alter renal function within 5 half-lives prior to the first dose of Cenderitide or placebo.
10.	Presence of cardiac lesions or comorbidities that may contraindicate the use of natriuretic peptides, such as clinically significant cardiac valvular stenosis, hypertrophic cardiomyopathy, restrictive cardiomyopathy, constrictive pericarditis, primary pulmonary hypertension, or uncorrected congenital heart disease that contraindicates the use of vasodilators.
11.	History of blood pressure >190/115 mmHg or unexplained syncope within the past 3 months.
12.	Symptomatic carotid artery disease, known critical carotid stenosis, or stroke within the past 3 months.
13.	Clinically significant renal artery stenosis.
14.	Baseline hemoglobin <10.0 g/dl.
15.	Serum sodium <130 mEq/L, potassium <3.6 mEq/L, or magnesium <1.7 mEq/L.
16.	Elevated aspartate aminotransferase (AST) or alanine aminotransferase (ALT) at least 5 times the upper limit of normal or bilirubin at least 3 times the upper limit of normal.
17.	Creatinine clearance (CrCl) <50 ml/min/1.73m^2, as calculated by Cockcroft-Gault formula and adjusted for body surface area within the past year or at screening, or requirement for dialysis.
18.	History of alcohol abuse within the past 6 months.
19.	Consumption of a phosphodiesterase-5 inhibitor (sildenafil, vardenafil, or tadalafil) within 72 h of receiving Cenderitide or placebo.
20.	Inability to communicate effectively with study personnel.
21.	Body mass index (BMI) >38 kg/m^2.

also observed a signal of possible GFR-enhancing properties in subjects with mild renal insufficiency. Overall, the results of this study encourage further investigations of Cenderitide in stable chronic HF as a potential innovative strategy to enhance cardiorenal function and preserve cardiorenal structure.

METHODS

Study design

This human study was designed as a randomized, double-blind, placebo-controlled trial to be conducted in subjects with stable chronic HF and reduced ejection fraction (EF) which was performed at the Clinical Research Unit (CRU) of the Mayo Clinic Center for Translational Science Activities (CTSA), Mayo Clinic, Rochester, MN. The study was supported by the National Heart, Lung, and Blood Institute (NHLBI). All subjects provided written, informed consent before enrollment. Study documentation was reviewed and approved by the Ethics Committee of the Mayo Clinic. The study was conducted in accordance with the ethical principles of the Declaration of Helsinki and its amendments, the US Food and Drug Administration Principles of Good Clinical Practice, and International Conference on Harmonization Guidelines, where applicable. The study was registered at ClinicalTrials.gov (ID: NCT00620308).

Eighteen subjects were randomized in a 2:1 ratio to Cenderitide vs. placebo to confirm the safety and the pharmacodynamics of Cenderitide in stable HF. Experimental periods included 1-h baseline and 4-h intravenous infusion of Cenderitide at 20 ng/kg/min based on previous studies in experimental animals and the first-in-human study in normal volunteers.[5,19]

Patients

Main inclusion criteria were as follows: 1) male, postmenopausal female, or surgically sterilized female; 2) age 21 years or older and able to give written informed consent; 3) NYHA functional class I–III; and 4) LVEF $\leq$40% documented within the last 2 years. The key exclusion criteria are listed in **Table 3**.

Screening

Screening was done prior to commencement of the study by medical history, physical examination, and screening laboratory tests including echocardiogram for LVEF. Eighteen HF patients who were assessed according to the inclusion/exclusion criteria were stabilized for $\geq$1 week on a no-add-salt diet (120 mEq = sodium per day) and admitted to the CRU of the Mayo Clinic CTSA on the day prior to the study.

Eligible patients were maintained on stable doses of HF medications, such as diuretics, angiotensin converting enzyme (ACE) inhibitors, angiotensin receptors blockers (ARBs), beta-blockers, aldosterone antagonists, or digoxin, for 1 week prior to the day of the study. Diuretics were on hold following the last dose on the day prior to the acute study until completion of the study. An ultrasound scan of the bladder was performed to assess complete emptying of the bladder. If a significant amount of residual urine was present, informed consent for the use of a urinary catheter during the study was obtained from the subject.

Randomization to double-blind treatment

Random treatment assignments were made by the use of a randomization code that was sent directly from the study statistician to the Mayo Research Pharmacy. The study pharmacist, who was not involved in data analysis, assisted in the preparation of Cenderitide and placebo (dextrose 5% in water, D5W). HF patients were randomized to placebo (D5W) or Cenderitide (20 ng/kg/min) for 4-h intravenous infusion. The dosing regimen was derived following consideration of *in vivo* experimental data from the first-in-human normal volunteer study.[19]

Monitoring of safety, tolerability, and cardiovascular function

The study consisted of 5 h and included a 1-h baseline and a 4-h period of study drug or placebo infusion. Blood and urine sampling and noninvasive hemodynamic parameters were collected during each period. Automated blood pressure measurements were obtained once every 15 min. Heart rate was monitored continuously by ECG. Following infusion of the study drug, the subjects were observed for 24 h in the CRU. Subjects were assessed clinically for any adverse reactions. If there were no concerns, the subjects were dismissed from the CRU.

Assessment of neurohumoral function

Plasma and urinary cGMP and plasma CNP to determine Cenderitide immunoreactivity were performed with previously reported methods.[5,29,30] Angiotensin II and aldosterone were measured as previously reported.[31,32]

Assessment of renal hemodynamics and function

Prior to receiving Cenderitide, all patients received infusion of iothalamate for 2 h for measurement of GFR.[33] The first hour was an equilibration period and the second hour was the baseline for comparison. This was followed by a 4-h collection period of urine and blood sampling during placebo or Cenderitide infusion. In *post-hoc* analysis, patients in the Cenderitide group were divided into two groups using the median baseline GFR (65 ml/min/1.73m^2) as the cutoff value: 1) Cenderitide with low-baseline GFR group ($n = 6$), and 2) Cenderitide with high-baseline GFR group ($n = 6$) to also assess GFR responses.

Data analysis

Statistical analysis was performed using JMP software v. 13 (SAS Institute, Cary, NC). Continuous variables were expressed as median and interquartile range and were compared using an unpaired *t*-test for equal variance data and Welch's test for nonequal variance data. The Wilcoxon rank-sum test was performed for nonnominal distributed data. Categorical variables were compared using chi-square test or Fisher's exact test. Two-way analysis of variance (ANOVA) was used to compare the means of these parameters between groups, followed by the Bonferroni posttest, where applicable. The stratification of patients based on GFR was an *ad-hoc* analysis. $P < 0.05$ was considered statistically significant.

ACKNOWLEDGMENTS

This study was supported by grants from the National Institutes of Health: PO1 HL76611 (JCB) and RO1 HL36634 (JCB) and from the ASCPT (CYWL). We also acknowledge support from the Mayo Clinic NIH supported CTSA (UL1TR000135).

CONFLICT OF INTEREST

Dr. Burnett is an inventor of Cenderitide.

AUTHOR CONTRIBUTIONS

K.W.W., D.M.S., B.Q.D., B.K., L.C., and J.A.J. wrote the article; K.W.W., A.R.E, B.B., Y.G., T.A.H., B.K., T.L, C.W.M., B.S., T.T.V., and J.A.J. designed the research; K.W.W., D.M.S., A.R.E., T.A.H., B.K., B.S., T.T.V., and D.T.W. performed the research; K.W.W., D.M.S., B.Q.D., Y.G., C.W.M., D.T.W., L.C., and J.A.J. analyzed the data; M.C-S., T.L., and D.T.W. contributed new reagents/analytical tools.

1. Meems, L.M.G. & Burnett, J.C. Jr. Innovative therapeutics: designer natriuretic peptides. *JACC Basic Transl. Sci.* **1**, 557–567 (2016).
2. Marso, S.P. *et al*. Liraglutide and cardiovascular outcomes in type 2 diabetes. *N. Engl. J. Med.* **375**, 311–322 (2016).
3. Rizzuti, M., Nizzardo, M., Zanetta, C., Ramirez, A. & Corti, S. Therapeutic applications of the cell-penetrating HIV-1 Tat peptide. *Drug Discov. Today.* **20**, 76–85 (2015).
4. Teerlink, J.R. *et al*. Serelaxin, recombinant human relaxin-2, for treatment of acute heart failure (RELAX-AHF): a randomised, placebo-controlled trial. *Lancet* **381**, 29–39 (2013).
5. Lisy, O., Huntley, B.K., McCormick, D.J., Kurlansky, P.A. & Burnett, J.C. Jr. Design, synthesis, and actions of a novel chimeric natriuretic peptide: CD-NP. *J. Am. Coll. Cardiol.* **52**, 60–68 (2008).
6. Dickey, D.M., Burnett, J.C. Jr. & Potter, L.R. Novel bifunctional natriuretic peptides as potential therapeutics. *J. Biol. Chem.* **283**, 35003–35009 (2008).
7. Waldman, S.A., Rapoport, R.M. & Murad, F. Atrial natriuretic factor selectively activates particulate guanylate cyclase and elevates cyclic GMP in rat tissues. *J. Biol. Chem.* **265**, 14332–14334 (1984).
8. Potter, L.R., Abbey-Hosch, S. & Dickey, D.M. Natriuretic peptides, their receptors, and cyclic guanosine monophosphate-dependent signaling functions. *Endocr. Rev.* **27**, 47–72 (2006).
9. Koller, K.J. *et al*. Selective activation of the B natriuretic peptide receptor by C-type natriuretic peptide (CNP). *Science* **252**, 120–123 (1991).
10. Suga, S. *et al*. Receptor selectivity of natriuretic peptide family, atrial natriuretic peptide, brain natriuretic peptide, and C-type natriuretic peptide. *Endocrinology* **130**, 229–239 (1992).
11. Lee, C.Y.W. *et al*. Cenderitide: Structural requirements for the creation of a novel dual particulate guanylyl cyclase receptor agonist with renal enhancing in vivo and ex vivo actions. *Eur. Heart J. Cardiovasc. Pharmacother.* **2**, 98–105 (2016).
12. Wojta, J. Cenderitide: a multivalent designer-peptide-agonist of particulate guanylyl cyclase receptors with considerable therapeutic potential in cardiorenal disease states. *Eur. Heart J. Cardiovasc. Pharmacother.* **2**, 106–107 (2016).
13. Kenny, A.J. & Stephenson, S.L. Role of endopeptidase-24.11 in the inactivation of atrial natriuretic peptide. *FEBS Lett.* **232**, 1–8 (1988).
14. Lisy, O. *et al*. Renal actions of synthetic Dendroaspis natriuretic peptide. *Kidney Int.* **56**, 502–508 (1999).
15. Singh, G. *et al*. Characterization of the snake venom ligand [125I]-DNP binding to natriuretic peptide receptor-A in human artery and potent DNP mediated vasodilatation. *Br. J. Pharmacol.* **149**, 838–844 (2006).
16. Dickey, D.M. & Potter, L.R. Dendroaspis natriuretic peptide and the designer natriuretic peptide, CD-NP, are resistant to proteolytic inactivation. *J. Mol. Cell. Cardiol.* **51**, 67–71 (2011).
17. Ichiki, T. *et al*. Cardiac fibrosis in end-stage human heart failure and the cardiac natriuretic peptide guanylyl cyclase system: regulation and therapeutic implications. *J. Mol. Cell. Cardiol.* **75**, 199–205 (2014).
18. Martin, F.L. *et al*. CD-NP: a novel engineered dual guanylyl cyclase activator with anti-fibrotic actions in the heart. *PLoS One* **7**, e52422 (2012).
19. Lee, C.Y.W. *et al*. Pharmacodynamics of a novel designer natriuretic peptide, CD-NP, in a first-in-human clinical trial in healthy subjects. *J. Clin. Pharmacol.* **49**, 668–673 (2009).
20. Travers, J.G., Kamal, F.A., Robbins, J., Yutzey, K.E. & Blaxall, B.C. Cardiac fibrosis: the fibroblast awakens. *Circ. Res.* **118**, 1021–1040 (2016).
21. Bonnans, C., Chou, J. & Werb, Z. Remodeling the extracellular matrix in development and disease. *Nat. Rev. Mol. Cell. Biol.* **15**, 786–801 (2014).
22. Horio, T. *et al*. Gene expression, secretion, and autocrine action of C-type natriuretic peptide in cultured adult rat cardiac fibroblasts. *Endocrinology* **144**, 2279–2284 (2003).
23. Soeki, T. *et al*. C-type natriuretic peptide, a novel antifibrotic and antihypertrophic agent, prevents cardiac remodeling after myocardial infarction. *J. Am. Coll. Cardiol.* **45**, 608–616 (2005).
24. Damman, K. & Testani, J.M. The kidney in heart failure: an update. *Eur. Heart J.* **14**, 1437–1444 (2015).
25. Metra, M. *et al*. Is worsening renal function an ominous prognostic sign in patients with acute heart failure? The role of congestion and its interaction with renal function. *Circ. Heart Fail.* **5**, 54–62 (2012).
26. McMurray, J.J. *et al*. Angiotensin-neprilysin inhibition versus enalapril in heart failure. *N. Engl. J. Med.* **371**(11), 993–1004 (2014).
27. Chen, H.H., Lainchbury, J.G., Harty, G.J. & Burnett, J.C. Jr. Maximizing the natriuretic peptide system in experimental heart failure: subcutaneous brain natriuretic peptide and acute vasopeptidase inhibition. *Circulation* **105**(8), 999–1003 (2002).
28. Chen, H.H. *et al*. Novel protein therapeutics for systolic heart failure: chronic subcutaneous B-type natriuretic peptide. *J. Am. Coll. Cardiol.* **60**, 2305–2312 (2012).
29. Wan, S.H. *et al*. Chronic peptide therapy with B-type natriuretic peptide in patients with pre-clinical diastolic dysfunction (Stage B Heart Failure). *JACC Heart Fail.* **4**(7), 539–547 (2016).
30. Sangaralingham, S.J. *et al*. Circulating C-type natriuretic peptide and its relationship to cardiovascular disease in the general population. *Hypertension* **65**, 1187–1194 (2015).
31. Luchner, A. *et al*. Angiotensin II in the evolution of experimental heart failure. *Hypertension* **28**, 472–477 (1996).
32. Buglioni, A. *et al*. Circulating aldosterone and natriuretic peptides in the general community: relationship to cardiorenal and metabolic disease. *Hypertension* 2015; **65**(1), 45–53 (2015).
33. Dowling, T.C., Frye, R.F. & Zemaitis, M.A. Simultaneous determination of p-aminohippuric acid, acetyl-p-aminohippuric acid and iothalamate in human plasma and urine by high-performance liquid chromatography. *J. Chromatogr. B Biomed. Sci. Appl.* **716**, 305–313 (1998).