**PRECLINICAL RESEARCH**

# Design, Synthesis, and Actions of a Novel Chimeric Natriuretic Peptide: CD-NP

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**Objectives**

Our aim was to design, synthesize and test in vivo and in vitro a new chimeric peptide that would combine the beneficial properties of 2 distinct natriuretic peptides with a biological profile that goes beyond native peptides.

**Background**

Studies have established the beneficial vascular and antiproliferative properties of C-type natriuretic peptide (CNP). While lacking renal actions, CNP is less hypotensive than the cardiac peptides atrial natriuretic peptide and B-type natriuretic peptide but unloads the heart due to venodilation. *Dendroaspis* natriuretic peptide is a potent natriuretic and diuretic peptide that is markedly hypotensive and functions via a separate guanylyl cyclase receptor compared with CNP.

**Methods**

Here we engineered a novel chimeric peptide CD-NP that represents the fusion of the 22-amino acid peptide CNP together with the 15-amino acid linear C-terminus of *Dendroaspis* natriuretic peptide. We also determined in vitro in cardiac fibroblasts cyclic guanosine monophosphate-activating and antiproliferative properties of CD-NP.

**Results**

Our studies demonstrate in vivo that CD-NP is natriuretic and diuretic, glomerular filtration rate enhancing, cardiac unloading, and renin inhibiting. CD-NP also demonstrates less hypotensive properties when compared with B-type natriuretic peptide. In addition, CD-NP in vitro activates cyclic guanosine monophosphate and inhibits cardiac fibroblast proliferation.

**Conclusions**

The current findings advance an innovative design strategy in natriuretic peptide drug discovery and development to create therapeutic peptides with favorable properties that may be preferable to those associated with native natriuretic peptides. *(J Am Coll Cardiol 2008;52:60–8) © 2008 by the American College of Cardiology Foundation*

An emerging innovative therapeutic strategy is the engineering of proteins, which fuses structural elements unique for 1 peptide with active structures from separate peptides to create chimeras, which possess attractive therapeutic properties (1). Such an approach with natriuretic peptides is advanced in the current study and involves the selection and incorporation of isolated structural determinants from 2 native peptides that result in a unique peptide with a specific activity profile that could be more useful than their natural counterparts as therapeutic agents in cardiorenal disease.

C-type natriuretic peptide (CNP) is a 22-amino acid (AA) peptide that shares structural homology with other members of the natriuretic peptide family, which includes the cardiac hormones atrial natriuretic peptide (ANP) and B-type natriuretic peptide (BNP), all of which function via well-characterized particulate guanylyl cyclase receptors (i.e., NPR-A for ANP and BNP; NPR-B for CNP) and the second messenger cyclic 3′5′ guanosine monophosphate (cGMP) (2–4). While possessing structural similarity, CNP is genetically distinct from ANP and BNP. Also unlike ANP or BNP, CNP lacks a COOH-terminus (C-terminus) AA extension, which may explain, in part, its lack of natriuretic properties (5,6). Since its discovery in 1990, we have learned that CNP is principally an endothelial cell-derived peptide (7–13). In isolated venous and arterial rings, CNP activates NPR-B receptors in veins while ANP and BNP bind to NPR-A receptors in both arteries and veins.
which is consistent with the less hypotensive actions of CNP as compared with ANP and BNP (14–16).

In addition to preferential venodilating properties, studies have also reported that CNP possesses more potent anti-proliferative and collagen-suppressing properties in cardiac fibroblasts (CFs) as compared with ANP and BNP (11). Relevant to such fibro-inhibiting properties, studies have shown that 14 days of continuous infusion of CNP in rodents with acute myocardial infarction (AMI) markedly attenuates ventricular dilation, cardiac fibrosis, and cardiomyocyte hypertrophy (17). The chronic infusion of CNP was without any hypotensive actions.

In clear contrast to ANP and BNP, CNP lacks significant natriuretic and diuretic actions when infused into humans. This may explain its lack of utility in sodium- and water-retaining syndromes such as acute heart failure (AHF) despite its attractive venodilating and antifibrotic properties (15,16).

In 1992, Schweitz et al. (18) reported the in vitro biological actions of a newly discovered peptide Dendroaspis natriuretic peptide (DNP). We subsequently reported that DNP, which was originally isolated from the green mamba, was in vivo potently natriuretic and diuretic and possessed cardiac unloading actions but with significant hypotensive properties (19,20). In the original report of the vasoactive actions of DNP, Schweitz et al. (18) concluded that DNP, like ANP and BNP, functions via the NPR-A receptor as saturation with ANP markedly attenuated the cGMP-activating actions of DNP in cultured human endothelial cells. Indeed, the in vivo actions of DNP that include natriuresis and hypotension are consistent with NPR-A activation as such effects closely mimic the properties of ANP and BNP but not CNP. Indeed, Singh et al. (21) recently have reported that DNP has a higher affinity for the NPR-A receptor in human myocardium as compared with ANP and BNP.

A key structural feature of DNP is that it possesses the longest C-terminus of the known natriuretic peptides consisting of 15 AA as compared with 5 AA for ANP, 6 AA for BNP and none for CNP. Indeed, the long C-terminus of DNP may render DNP highly resistant to degradation by neutral endopeptidase (NEP) contributing to potent natriuretic and diuretic actions (22). Further, the lack of a C-terminus for CNP may explain the observation that of the 3 known endogenous natriuretic peptides CNP is the most susceptible to NEP degradation, which could limit its renal actions as NEP is expressed greatest in the kidney (23).

Based upon exploratory studies of the 15-AA C-terminus of DNP, we hypothesized that fusion of the 15-AA C-terminus of DNP into the C-terminus position of the core 22-AA ring structure of CNP would result in a synthetic chimeric peptide that in vivo would possess the cardiac unloading actions of CNP with minimal hypertensive properties together with the additional renal effects of natriuresis and diuresis. We also hypothesized that this chimera called CD-NP would retain properties of CNP in vitro in activating cGMP in CFs and inhibiting cell proliferation. Thus, we report the design, protein synthesis, and biological actions of a chimeric natriuretic peptide with potentially beneficial efficacy and safety for the treatment of cardiorenal diseases states such as AHF and AMI.

**Methods**

We synthesized 2 peptides based upon the known sequence of DNP and CNP. First we synthesized the linear 15-AA C-terminus sequence (PSLRDPNAPNSTSA) of DNP (Fig. 1). We call this peptide “C-terminus.” Secondly, we designed and synthesized a chimera of CNP and the C-terminus of DNP (GLSKGCFLKL-DRIGSMGGLCPSLRDPNAPNSTSA), which is a 37-AA peptide that we call “CD-NP.” These 2 peptides were synthesized using solid phase methods by the Mayo Protein Core Facility on an ABI 431A Peptide Synthesizer (PE Biosystems, Foster City, California) on a pre-loaded Wang resin with N-Fmoc-L-amino acids (SynPep, Dublin, California). Coupling of each AA to the resin-linked peptide was performed in 1-methyl-2-pyrrolidinone (NMP) for 40 min. Each Fmoc AA was activated in a solution of 1-hydroxybenzotriazole in NMP for 30 min. Deprotection of the Fmoc protecting group was performed with 20% piperidine in NMP for 20 min before coupling of the next activated AA. Subsequently, peptides were deprotected and removed from the resin by treatment with a mixture of 82.5% trifluoroacetic acid/5% water/5% thioanisole/2.5% ethanedithiol/5% phenol for 2 h at room temperature. Each peptide was washed by precipitation in 3 × 50 ml of cold methyl t-butyl ether and purified by reverse phase high performance liquid chromatography on a Jupiter C18 column (Phenomenex, Torrance, California) in 0.1% trifluoroacetic acid/water with a gradient of 10% to 70% B in 50 min.

The identity of each peptide was verified by electrospray ionization mass analysis on a Perkin/Elmer Sciex API 165 Mass Spectrometer (PE Biosystems). Disulfide bridges in CD-NP were formed by overnight air oxidation in a 50 mM ammonium bicarbonate pH 8.5 buffer.

**Integrative in vivo biological actions of C-terminus and CD-NP in normal canines.** Experiments were performed in 5 separate groups of normal anesthetized dogs. All studies conformed to the guidelines of the American Physiological

**Abreviations and Acronyms**

- **AA** = amino acid
- **AHF** = acute heart failure
- **AMI** = acute myocardial infarction
- **ANP** = atrial natriuretic peptide
- **BNP** = B-type natriuretic peptide
- **BrdU** = bromodeoxyuridine
- **CF** = cardiac fibroblast
- **cGMP** = cyclic 3’5’ guanosine monophosphate
- **CNP** = C-type natriuretic peptide
- **DNP** = Dendroaspis natriuretic peptide
- **GFR** = glomerular filtration rate
- **NEP** = neutral endopeptidase
- **NMP** = 1-methyl-2-pyrrolidinone
- **PFRN** = proximal fractional sodium reabsorption
- **PRA** = plasma renin activity

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Society and were approved by the Mayo Clinic Animal Care and Use Committee. On the evening before the experiments, 300 mg of lithium carbonate was administered orally for the assessment of segmental renal tubular function and then dogs were fasted overnight. On the day of the acute experiment, all dogs were anesthetized with pentobarbital sodium given intravenously (30 mg/kg). Dogs were mechanically ventilated (Harvard respirator, Harvard Apparatus, Millis, Massachusetts) with 4 l/min of supplemental oxygen. A left lateral flank incision was made, and the left kidney was exposed. The ureter was cannulated for timed urine collection, and a calibrated electromagnetic flow probe was placed around the left renal artery and connected to a flowmeter (model FM 5010, Carolina Medical, King, North Carolina) for monitoring of renal blood flow. Finally, the right femoral vein was cannulated with 2 polyethylene catheters (PE-240), 1 for infusion of inulin and the other for the infusion of C-terminus. The right femoral artery was cannulated for direct arterial blood pressure measurement and arterial blood sampling. For determination of cardiac filling pressures in those groups that received CD-NP or measurement of cardiac output, a Swan-Ganz catheter (Edwards, Mountain View, California) was inserted into the right internal jugular vein.

After completion of the surgical preparation, a priming dose of inulin (ICN Biomedicals, Cleveland, Ohio) was injected, followed by a constant infusion of 1 ml/min. The dogs were allowed to equilibrate for 60 min without intervention. After an equilibration period of 60 min, a 30-min baseline clearance (Baseline) was performed. This was followed by a 15-min lead-in period during which C-terminus infusion at 42 ng/kg/min in the first group (n = 6) was begun intravenously after which the second 30-min clearance (C-terminus) period was performed. A time control with intravenous saline at 1 ml/min (n = 6) was performed to serve as a control for the CD-NP group. During each clearance, renal hemodynamic and excretory response was recorded. In the third group, CD-NP was infused at 3 concentrations that were 10, 50, and 100 ng/kg/min each for 30 min with a 15-min lead-in period for each dose. Groups 4 (n = 6) and 5 (n = 6) received an equimolar concentration of 10 and 50 ng/kg/min of CD-NP or BNP, respectively.

Plasma and urine electrolytes including lithium were measured by flame-emission spectrophotometer (IL943, Flame Photometer, Instrumentation Laboratory, Lexington, Massachusetts). Plasma and urine inulin concentrations were measured by the anthrone method, and glomerular filtration rate (GFR) was measured by the clearance of inulin. The lithium clearance technique was employed to estimate the proximal fractional sodium reabsorption (PFRNa) and distal fractional sodium reabsorption. Proximal fractional sodium reabsorption was calculated by the following formula: 

\[ \frac{1}{\text{lithium clearance/GFR}} \times 100. \]

Distal fractional sodium reabsorption was calculated by the formula: 

\[ \frac{\text{lithium clearance} - \text{sodium clearance}}{\text{lithium clearance}} \times 100. \]

Plasma and urinary cGMP and plasma renin activity (PRA) were measured by commercially available radioimmunoassay as described previously.

In vitro studies on CD-NP cGMP generation and inhibition of cell proliferation in human CFs. Studies were performed in human CFs (ScienCell, San Diego, California) as we have previously reported (24). Only cell passages 1 through 4 were used for experiments. Cells were exposed to CD-NP (10^{-11} to 10^{-6} M) for 15 min to determine cGMP activation. The samples were assayed using a competitive radioimmunoanalysis cGMP kit (Perkin-Elmer, Boston, Massachusetts). Briefly, samples and standards were incubated with anti-human cGMP polyclonal antibody and ^{125}\text{-antigen for 18 h. Cyclic GMP assay buffer was added to the samples, and they were}
centrifuged for 20 min at 2,500 rpm. The free fraction was aspirated off, and the bound fraction was counted and concentrations determined. Values are expressed as pmoles/ml. There was no cross-reactivity with ANP, BNP, CNP, and endothelin, and <0.001% cross-reactivity with cyclic adenosine monophosphate, GMP, guanosine diphosphate, adenosine triphosphate, and guanosine triphosphate.

For CFs proliferation studies, 70% to 80% confluent cells at passage 1 to 4 cells were treated for 24 h with 10⁻⁸ M cardiotrophin-1 to induce cell proliferation. CD-NP at the concentration of 10⁻⁸ M was added to cardiotrophin-1-stimulated CFs to determine its effect on cell proliferation. Untreated fibroblasts were processed as a control. Colormetric bromodeoxyuridine (BrdU) cell proliferation enzyme-linked immunoadsorbent assay (Roche, Indianapolis, Indiana) was performed as directed. Briefly, CFs were labeled with BrdU for 2 h in a CO₂ 37°C incubator. Antibromodeoxyuridine was added and allowed to react for 90 min at room temperature. The anti-BrdU was removed, and the CFs were washed 3 times with a washing solution. Colormetric substrate solution was added, and color was allowed to develop for 30 min. Absorbance at 370 nm was measured on a SpectraMax spectrophotometer (Molecular Devices, Sunnyvale, California).

Statistical analysis. Results of quantitative studies are expressed as mean ± SE. Statistical comparisons within 1 group when comparing 2 clearances was performed using Student t test, and when comparing multiple clearances, repeated measures analysis of variance (ANOVA) followed by post hoc Dunnett’s test. Statistical comparison between groups was performed using 2-way ANOVA followed by Bonferroni post-test. Statistical significance was accepted for a p value <0.05.

Results

In vivo actions of C-terminus of DNP and CD-NP. The C-terminus of DNP that is lacking the core ring structure of DNP was natriuretic and diuretic (Fig. 2). We localized this natriuretic action to the proximal segment of the renal nephron as we observed a decrease in PFRNa suggesting that this part of the nephron contributes to the renal actions of this small peptide (Table 1). We noted no change in GFR, renal blood flow, or urinary cGMP excretion. Importantly, in a time control group that also received saline at 1 ml/min as in the C-terminus group, parameters of renal function were unchanged (data not shown).

Figure 3 reports the in vivo responses to CD-NP infusion. All 3 doses of CD-NP decreased pulmonary capillary wedge pressure while 2 doses decreased right atrial pressure. These cardiac unloading responses were associated with minimal decreases in mean arterial pressure even during the infusion of a high dose of CD-NP. We also observed potent natriuretic and diuretic responses with CD-NP in normal canines (Fig. 4). Specifically, both medium and high dose CD-NP increased urinary excretion of sodium and urine flow (Fig. 4). Despite a slight decrease in mean arterial pressure during the infusion of a high dose of CD-NP, GFR increased. There was no change in renal blood flow during CD-NP infusion (240 ± 40 ml/min [baseline] to 245 ± 27 ml/min [dose-10] to 248 ± 25 ml/min [dose-50] to 253 ± 22 ml/min [dose-100] and to 221 ± 32 ml/min [recovery]). Natriuresis and diuresis involved a decrease in proximal tubular reabsorption of sodium as PFRNa during the infusion of medium dose of CD-NP was reduced. In addition, a decrease in distal fractional sodium reabsorption during the infusion of medium and high doses of CD-NP was also observed, which indicates that CD-NP also involves activities in the terminal nephron (Fig. 5). These renal parameters returned to baseline levels after discontinuation of the infusion of CD-NP. These actions were also associated with suppression of PRA during the infusion of low and medium doses of CD-NP (Table 2). Plasma renin activity returned to baseline levels after discontinuation of infusion. Medium and high doses of CD-NP increased plasma and urinary cGMP (Table 2).

At a dose of 50 ng/kg/min, CD-NP significantly increased GFR in contrast to a lack of increase in GFR in the group receiving an equimolar dose of BNP (Fig. 6). Impor-
tantly, this dose of CD-NP was associated with a less hypotensive action than BNP.

**CD-NP–mediated cGMP generation in CFs and inhibition of proliferation.** Figure 7 reports findings from in vitro studies in cultured human CFs. These studies demonstrate that CD-NP activates cGMP in a dose-dependent manner (Fig. 7A). Further, when compared with CNP, BNP, and DNP, the cGMP generated with CD-NP was similar to CNP and significantly greater (p < 0.05) than DNP and BNP at 10^{-6} M (data not shown). We then sought to define if CD-NP possessed antiproliferative actions. We utilized the pro-fibrotic and hypertrophic cytokine cardiotrophin-1 that has been reported by others and ourselves as a markedly robust activator of CFs and which is activated in states such as AMI and heart failure (25–27). CD-NP suppressed cell proliferation in human CFs induced by cardiotrophin-1 as assessed by BrdU uptake as a measure of DNA synthesis and cellular proliferation (Fig. 7B).

**Discussion**

Here we report the design, protein synthesis, and in vivo cardiorenal actions of a chimeric natriuretic peptide that is a fusion of the core 22-AA ring structure of CNP together with the 15-AA C-terminus of DNP. Unlike CNP, which lacks significant renal actions, CD-NP is potently natriuretic and diuretic, GFR enhancing, and PRA inhibiting. CD-NP reduces cardiac filling pressures and when compared with BNP is less hypotensive. In vitro studies in cultured CFs establish that CD-NP, like CNP, activates cGMP and suppresses CF proliferation induced by cardiotrophin-1, which is known to be activated in heart failure and myocardial infarction. This modular design incorporating sequences of 2 native peptides thus emerges as attractive from a therapeutic standpoint as many of the current native natriuretic peptides for the treatment of AHF and AMI such as ANP and BNP optimize 1 function such as cardiac unloading while sacrificing another such as maintenance of blood pressure, which is overcome by CD-NP.

The current study also demonstrates that the chimeric peptide CD-NP, which combines the full-length 22 AA ring structure of CNP with the 15 AA C-terminus of DNP, is potently natriuretic and diuretic and GFR enhancing, which is in contrast to the reported lack of significant renal actions of CNP (5,15,16). The natriuretic action of CD-NP was localized to both the proximal and distal nephrons. Contributing to the natriuretic and diuretic action of CD-NP was an increase in GFR. The increase in GFR, therefore, must be mediated by a decrease in pre-glomerular vascular resistance together with an increase in post-glomerular vascular tone to increase glomerular capillary hydrostatic pressure while there was no significant change in renal blood flow. Alternatively, an increase in the glomerular ultrafiltration coefficient (Kf) could contribute.
to the GFR increase. Importantly, we observed that the renal hemodynamic and tubular actions of CD-NP were associated with increases in plasma and urinary cGMP concentrations consistent with particulate guanylyl cyclase receptor activation.

In the past, we and others have speculated that under physiologic condition renally synthesized CNP acts in an autocrine/paracrine fashion to activate NPR-B receptors, which are expressed in the kidney, resulting in natriuretic and diuretic actions (28–32). This would be in contrast to intravenously infused CNP, which lacks significant renal actions due to rapid degradation by renal NEP. It is tempting to speculate that the addition of the C-terminus of DNP protects CNP from degradation by NEP resulting in enhanced NPR-B activation by CD-NP that then could mediate increases in GFR together with natriuresis and diuresis. Of note, addition of AAs to the C-terminus of ANP has been demonstrated to increase ANP stability by increasing resistance to NEP degradation (33). This concept would be consistent with reports that inhibition of NEP during DNP administration potentiates the renal actions of DNP, which is observed during administration of native ANP or BNP together with NEP inhibition (22,34,35). It is also possible that this linear peptide (i.e., C-terminus of DNP) could retard the degradation of endogenous ANP or BNP by competing with NEP and/or reduce binding of the native natriuretic peptides to the natriuretic peptide clearance receptor (i.e., NPR-C) making more endogenous ANP and BNP available for NPR-A binding in the kidney. Finally, it is possible that the C-terminus of DNP also altered the ability of CNP to activate NPR-A directly, making CD-NP an NPR-B and NPR-A agonist. Clearly, CD-NP is natriuretic, diuretic, and GFR enhancing with less hypotensive actions but by mechanisms that will need to
be addressed in future studies. It should be noted that at the lower doses of CD-NP, PRA was suppressed. CD-NP unloading the normal heart with reductions in both right atrial pressure and pulmonary capillary wedge pressure. At the doses employed in the normal dogs, cardiac unloading occurred with no or only modest changes in arterial pressure. These are consistent with the conclusion that the reduction in cardiac filling pressures may involve reductions in cardiac pre-load via venodilation as occurs with CNP (14). Thus, CD-NP may address the need of a cardiac unloading natriuretic peptide with renal-enhancing actions with less hypotension than ANP or BNP, thus better maintaining renal function, which was the goal of our engineered chimeric peptide.

An important property of CNP and the natriuretic peptides is antifibrotic actions in the heart and other organs, which involve inhibition of fibroblast proliferation and suppression of collagen synthesis. Importantly, Horio et al. (11) demonstrated that CNP was more antifibrotic in cultured human CFs as compared with ANP or BNP. Such fibro-inhibiting actions have clinical relevance as demonstrated by studies of Soeki et al. (17) in which chronic CNP infusion in a rodent model of AMI for 14 days suppressed

Data are expressed as means ± SE. *p < 0.05 versus no treatment; **p < 0.05 versus CP-NP 10⁻¹¹M; †p < 0.05 versus CDNP 10⁻¹¹M. (B) Antiproliferative actions of CD-NP in human CFs. Bar graph reports colorimetric bromodeoxyuridine (BrdU) uptake in optical density units as measured by colorimetry. Data are expressed as means ± standard error. *p < 0.05 versus control. Cardiotrophin-1 = proliferation of fibroblasts by cardiotrophin-1; Cardiotrophin-1 + CD-NP = CD-NP added to cardiotrophin-1 stimulated fibroblasts; Control = untreated human cardiac fibroblasts.

Table 2 Neurohumoral Response to Infusion of CD-NP in Normal Dogs

<table>
<thead>
<tr>
<th></th>
<th>Baseline</th>
<th>CD-NP 10</th>
<th>CD-NP 50</th>
<th>CD-NP 100</th>
<th>Recovery</th>
</tr>
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<tbody>
<tr>
<td>PcGMP (pmol/ml)</td>
<td>9.4 ± 1.2</td>
<td>14.6 ± 1.6</td>
<td>30.3 ± 2.1*</td>
<td>50.1 ± 2.1*</td>
<td>32.7 ± 6.7*</td>
</tr>
<tr>
<td>UcGMPV (pmol/min)</td>
<td>1.648 ± 151</td>
<td>2.133 ± 234</td>
<td>4.566 ± 621*</td>
<td>9.999 ± 1.590*</td>
<td>6.661 ± 734*</td>
</tr>
<tr>
<td>PRA (ng/ml/hr)</td>
<td>8.2 ± 0.8</td>
<td>5.3 ± 1.1*</td>
<td>3.9 ± 0.7*</td>
<td>6.3 ± 1.1</td>
<td>9.0 ± 1.8</td>
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CD-NP 10 = infusion of 10 ng/kg/min of CD-NP; CD-NP 50 = infusion of 50 ng/kg/min of CD-NP; CD-NP 100 = infusion of 100 ng/kg/min (n = 8 except n = 6 for high dose); PcGMP = plasma cyclic 3’5’ guanosine monophosphate; PRA = plasma renin activity; UcGMPV = urinary cyclic 3’5’ guanosine monophosphate excretion.

Figure 6 Effect of CD-NP and Equimolar Doses of Human BNP in 2 Groups of Normal Dogs

Data are expressed as means ± SE. n = 6 in each group. *p < 0.05 versus baseline; †p < 0.05 between groups. Solid bars = CD-NP; open bars = B-type natriuretic peptide (BNP). Abbreviations as in Figures 3 and 4.

Figure 7 Effect of CD-NP on cGMP Generation and Antiproliferative Actions of CD-NP in Human CFs

(A) Cyclic 3’5’ guanosine monophosphate (cGMP) generation in human cardiac fibroblasts (CFs). Data are expressed as means ± SE. *p < 0.05 versus no treatment; **p < 0.05 versus CP-NP 10⁻¹¹M; †p < 0.05 versus CDNP 10⁻¹¹M. (B) Antiproliferative actions of CD-NP in human CFs. Bar graph reports colorimetric bromodeoxyuridine (BrdU) uptake in optical density units as measured by colorimetry. Data are expressed as means ± standard error. *p < 0.05 versus control. Cardiotrophin-1 = proliferation of fibroblasts by cardiotrophin-1; Cardiotrophin-1 + CD-NP = CD-NP added to cardiotrophin-1 stimulated fibroblasts; Control = untreated human cardiac fibroblasts.
ventricular remodeling and fibrosis with reductions in collagen III synthesis in the absence of reductions in blood pressure. In our in vitro studies in human CFs, we observed that CD-NP activated cGMP and possessed antiproliferative actions to cardiotropin-1, which is a potent pro-fibrotic factor that is known to be activated in heart failure and AMI (25–27). This action of CD-NP was demonstrated by inhibition of cardiotropin-1 augmentation of BrdU uptake. To further understand mechanisms by which CD-NP is antifibrotic, future studies will investigate collagen turnover in CFs in response to fibrogenic cytokines.

The natriuretic peptide BNP was approved in 2002 as a new drug for the treatment of acute decompensated heart failure based upon the randomized trial VMAC (The Vasodilation in the Management of Acute CHF) which demonstrated that, compared with nitroglycerin, BNP was superior in reducing cardiac filling pressures and compared with placebo was superior in improving dyspnea (36). With continued use of BNP, concerns were raised regarding renal function in human heart failure and even a continued use of BNP, concerns were raised regarding renal failure based upon the randomized trial VMAC (The Vasodilation in the Management of Acute CHF) which demonstrated that, compared with nitroglycerin, BNP was superior in reducing cardiac filling pressures and compared with placebo was superior in improving dyspnea (36). With continued use of BNP, concerns were raised regarding renal function with a key study reporting a lack of action of BNP on kidney function in human heart failure and even a worsening of renal function (37,38). As the level of GFR is increases in GFR and sodium excretion underscoring the importance of maintaining renal perfusion pressure in heart failure during infusion of natriuretic peptides (41). Indeed, in recent human studies in heart failure and during cardiac bypass surgery, low-dose BNP without hypotension has been demonstrated (42–44). Further, the chronic intermittent use of BNP in human heart failure was unassociated with any worsening of renal function compared with placebo. While further studies in disease states are warranted, CD-NP represents a novel therapeutic strategy, which possesses properties of a peptide-like BNP and ANP but with apparent less hypotension. The beneficial properties of CD-NP will be tested in future studies in models of experimentally induced heart failure.

Conclusions

In summary, we report the design, synthesis, and in vivo cardiorenal actions of a chimeric natriuretic peptide CD-NP that represents a fusion peptide of the full-length 22-AA structure of CNP together with the 15-AA C-terminus of DNP. Specifically, CD-NP is natriuretic and diuretic, GFR enhancing, renin inhibiting, and unloads the normal heart, with less hypotensive properties when compared with BNP. In addition, CD-NP in vitro possesses cGMP-activating and antiproliferative properties in CFs. As CD-NP contains the native CNP peptide, which activates NPR-B, the therapeutic potential of CD-NP is made highly relevant by recent reports that document the down-regulation of the NPR-A receptor in the heart and kidney in human and experimental heart failure (45,46). Our findings are significant and demonstrate the successful fusion of native CNP with an AA sequence taken from the C-terminus of DNP producing a chimera that now possesses clinically relevant renal actions with minimal hypotensive actions and favorable cardiovascular and humoral properties. These properties are especially attractive in acute cardiorenal syndromes such as AHF and AMI. Further studies in experimental models of cardiorenal disease and ultimately in human disease states will answer the question of whether this strategy will indeed yield new efficacious and safe drugs.

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Key Words: peptide ▪ kidney ▪ blood pressure ▪ cGMP ▪ sodium excretion ▪ fibroblasts.