Designer Natriuretic Peptides

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Abstract: Designer natriuretic peptides (NPs) are novel hybrid peptides that are engineered from the native NPs through addition, deletion, or substitution of amino acid(s) with a goal toward optimization of pharmacological actions while minimizing undesirable effects. In this article, selected peptides that were designed in our laboratory are reviewed, and future directions for research and development of designer NPs are discussed.

Key Words: natriuretic peptide, renal function, hybrid peptides

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INTRODUCTION

Designer natriuretic peptides (NPs) are novel hybrid peptides that are engineered from the native NPs through addition, deletion, or substitution of amino acid(s) (AA) with a goal toward optimization of pharmacological actions while minimizing undesirable effects. These strategies are often based on the integration of key structural determinants from the native NPs in activating the second messenger, cyclic guanosine monophosphate (cGMP), via the NP receptors (NPRs), resulting in renal, hemodynamic, and humoral effects that are characteristic of the native NPs. These designer peptides may exhibit improved pharmacological profiles as compared with the native peptides and may also possess unique biological actions that are not found in the native NPs.[1] In this article, we review 2 hybrid peptides that were designed in our laboratory in the past 15 years and discuss future directions for the research and development of novel designer NPs.

Native Natriuretic Peptides—The Backbone for Chimeric/Designer Peptides

Four mammalian NPs, atrial natriuretic factor (or atrial NP, ANP),[2] B-type NP (BNP),[3] C-type NP (CNP),[4] and urodilatin (URO),[5] have been identified and characterized (Fig. 1). In addition, *Dendroaspis* NP, from the green mamba snake (*Dendroaspis angusticeps*),[6] has been isolated and studied.[7–9] Atrial NP, BNP, URO, and *Dendroapsis* NP (DNP) are ligands for NPR-A, whereas CNP is the ligand for NPR-B.[6,10–13] Activation of NPR-A or NPR-B results in an increase in the second messenger, cGMP, which mediates favorable physiological responses in the cardiorenal axis and counteracts neurohumoral activation under pathophysiological conditions.[13]

Vasonatrin

Vasonatrin NP (VNP) was a synthetic NP that was designed in the Burnett Laboratory.[1] It was a 27-AA peptide, consisting of the full-length, 22-AA of human CNP and the 5-AA C-terminus of human ANP (Fig. 2).[1] In 1993, Wei et al[1] from our laboratory reported that VNP exerted venodilating actions like CNP and induced natriuresis like ANP but also possessed arterial vasodilating properties that were unique to VNP.[1] The rank order of vasorelaxation was observed to be VNP > ANP ≥ CNP in systemic arteries and VNP > CNP > ANP in systemic veins.[1] The vasorelaxant effect of VNP was independent of the endothelium.[1,14] In rats, VNP 50 μg/kg intravenously administered (IV) bolus increased plasma cGMP, urinary cGMP excretion, urine flow, and urinary sodium excretion, as well as decreased mean arterial pressure (MAP) and right atrial pressure, with enhanced natriuresis, diuresis, and urinary cGMP excretion in response to VNP versus CNP, but were less than those with ANP.[1] In autoradiographic studies, VNP was demonstrated to bind primarily to NPR-C in isolated rat and canine glomeruli and in canine femoral arteries.[15] In rat glomerular membranes, binding of VNP to NPR-A was demonstrated by cross-linking studies.[15] In isolated rat glomeruli, increase in cGMP production was induced by VNP (1 μM), suggesting NPR-A activation in the glomeruli.[15]

In isolated rat pulmonary artery, abdominal aorta, and celiac vein, the vasorelaxant effects of VNP have been demonstrated by Feng et al.,[16] with median effective concentrations of 16, 35, and 12 nmol/L, respectively. When compared with ANP and CNP, the rank orders of the vasorelaxant effects were VNP > ANP ≥ CNP for pulmonary artery, VNP > ANP > CNP for abdominal aorta, and VNP > CNP > ANP for celiac vein.[16] The vasorelaxant effects of VNP were similar regardless of the presence or absence of the endothelium.[16] In addition, this vasorelaxant effect has also been observed in human intramammary artery in a dose-dependent and endothelium-independent manner.[17]

In neonatal rat cardiac fibroblasts, VNP has been reported to attenuate hypoxia-induced, growth-promoting effects and increase in intracellular Ca^{2+}.[16,18] In pulmonary arterial smooth muscle cells, VNP inhibited proliferation to a greater extent than ANP and CNP.[19]

CD-NP

More recently, a novel chimeric NP, named CD-NP, was designed by Lisy et al in our laboratory.[20] CD-NP consists of the full-length 22 AAs of human CNP and the 15-AA C-terminus of DNP (Fig. 3). The rationale for the design of CD-NP was to transform CNP into a CNP-like peptide with enhanced renal actions without inducing systemic hypotension, as the latter is a clinically important side effect with the use of recombinant ANP[21] and BNP,[22] and in the clinical development of URO.[23,24] C-type NP, an NP of endothelial cell origin, was selected for this modular drug design, as CNP has been demonstrated to exert

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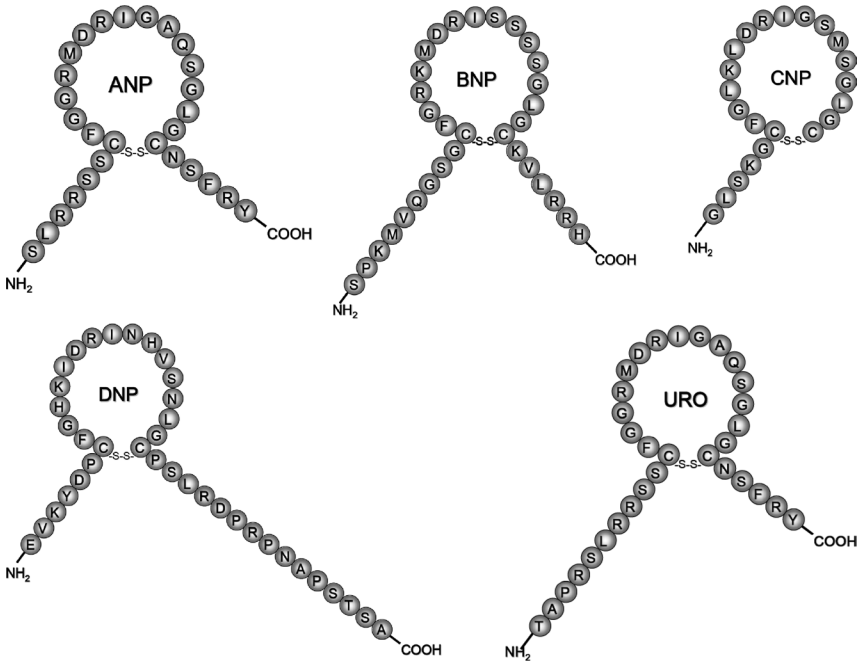

FIGURE 1. Amino acid structures for atrial natriuretic peptide (ANP), B-type natriuretic peptide (BNP), C-type natriuretic peptide (CNP), *Dendroaspis* natriuretic peptide (DNP), and urodilatin (URO).

predominantly venodilating effects[25] and thus is less likely to result in systemic hypotension.[13] The avoidance of excessive hypotension is an important consideration in the design of novel peptides to preserve renal perfusion pressure and renal function.[26,27] In addition, CNP possesses antiproliferative effects,[28] which is a highly desirable property for novel cardiovascular drugs against hypertrophy and remodeling. However, an important limitation of CNP is that it exerts only modest renal actions,[29] whereas DNP is potently natriuretic and diuretic, but it induces significant hypotension.[7,8] Thus, CD-NP was synthesized with the goal of combining the above complementary profiles of CNP and DNP into a single chimeric NP with renal-enhancing actions without inducing excessive hypotension.

In normal anesthetized dogs, CD-NP activates cGMP and exerts natriuretic, diuretic, and cardiac unloading and renin-angiotensin–suppressing actions with minimal effects on systemic blood pressure.[30,31] When compared with conventional therapy (BNP) on an equimolar basis, Lisy et al.[32] demonstrated that CD-NP 50 ng/kg per minute IV exerted significantly less effect on systemic blood pressure and was associated with a greater increase in glomerular filtration rate. When compared with an equimolar dose of CNP, Lee et al.[31] demonstrated that CD-NP 50 ng/kg per minute IV elicited greater increases in plasma cGMP, urinary cGMP excretion, and net renal generation of cGMP, which was associated with enhanced natriuresis. Moreover, CD-NP, not CNP, enhanced glomerular filtration rate and suppressed plasma renin activity and angiotensin II.[31] Taken together, these findings suggest that CD-NP represents a successful transformation of CNP to a CNP-like peptide with enhanced renal and neurohumoral actions.

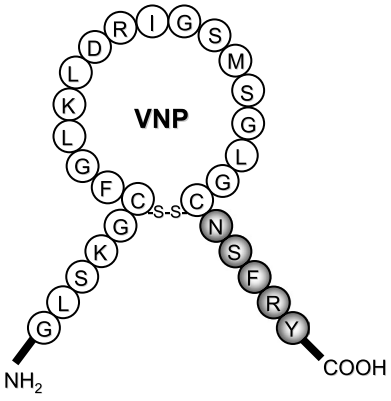

FIGURE 2. Amino acid structures for vasonatrin (VNP).

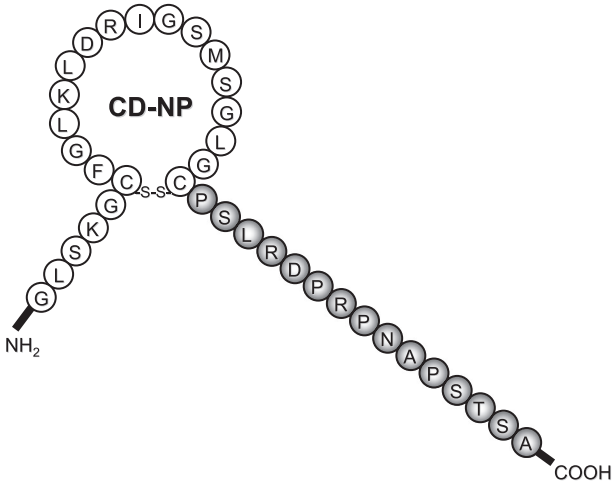

FIGURE 3. Fusion peptide of C-type natriuretic peptide and C-terminus of *Dendroapsis* natriuretic peptide (CD-NP).

In vitro, CD-NP stimulated cGMP production in cultured human cardiac fibroblasts to a greater extent than equimolar concentrations of BNP and DNP (10^{-6} mol/L).[20] CD-NP also exerted antiproliferative actions in cultured human cardiac fibroblasts that were treated with CT-1 to induce cell proliferation.[20] Moreover, CD-NP (10^{-6} mol/L), when compared with an equimolar concentration of CNP, stimulated an 8-fold greater cGMP response, which was attenuated by an NPR-A antagonist, suggesting involvement of NPR-A in mediating the cGMP response.[33] In comparison, the actions of CD-NP in human cardiac fibroblasts, in which NPR-B is expressed,[34] would be consistent with NPR-B activation by CD-NP. Thus, CD-NP may represent a novel chimeric NP that targets both NPR-A and NPR-B.

Following toxicology studies in rats and in dogs and subsequent regulatory approval, the first-in-human clinical trial on CD-NP was recently conducted.[35] This first-in-human clinical trial consisted of 2 stages: an open-label sequential dose escalation study (stage 1) and a randomized, double-blind, placebo-controlled study (stage 2).[35] In the first stage, 3 cohorts with a total of 12 subjects (4 subjects per cohort) were enrolled in the dose-escalation study. In stage 2, 10 subjects were randomized in the double-blind study, which evaluated the maximum tolerated dose (as determined in the first stage) of CD-NP versus placebo as a 4-hour continuous infusion. In the dose-escalation study, significant increases in plasma cGMP were observed when CD-NP was infused at 10, 17.5, and 25 ng/kg per minute IV.[35] Symptomatic orthostatic hypotension was observed in 2 subjects at the latter dose. The maximum tolerated dose was subsequently determined to be 17.5 ng/kg per minute and confirmed.[35] In the second stage, CD-NP significantly increased plasma cGMP, urinary cGMP excretion, and urinary sodium excretion, as compared with placebo.[35] Urine flow increased significantly in the CD-NP group versus baseline, whereas no significant increases were observed in the placebo group.[35] Glomerular filtration rate was preserved in both groups without a significant between-group difference in MAP, despite a slight decrease in MAP in the CD-NP group from baseline: (mean ± SEM) 85 ± 2 mm Hg to the end of infusion 82 ± 3 mm Hg ($P < 0.05$). No severe adverse events were reported. Overall, the favorable cGMP-activating, renal and hemodynamic effects of CD-NP that were observed in experimental studies[30,31] were indeed demonstrated for the first time in humans. The in vivo renal mechanisms of action of CD-NP and the therapeutic potential of CD-NP in human heart failure and the acute coronary syndromes are being explored in multicenter clinical trials.

Future Directions

As highlighted in a recent review by Letts and Loscalzo,[36] "chimeric molecules are single-chemical entities that possess at least 2 separate functions. In the design of new chimeric medicines, the 2 biologic actions are often designed to be synergistic[37] and thereby complement each other in activating a specific target, such as a gene, a receptor or an enzyme." Our work, to date, has demonstrated that novel designer NPs preserve favorable cardiorenal and neurohumoral effects of the native NPs while minimizing systemic hypotension, thus holding promise as a new generation of therapeutics for the prevention and treatment of various cardiorenal disease states. In particular, based on the favorable pharmacological profiles of the native NPs, it is anticipated that novel designer NPs may have potential applications in ischemic heart disease,[38] post-infarction remodeling,[28,39–41] peripheral vascular disease,[42] chronic heart failure,[43] hypertension,[44,45] metabolic diseases,[46,47] and cancer.[48–50] As potential therapeutic applications of the NPs continue to expand from acute to chronic diseases, there is an increasing need for novel strategies to optimize chronic peptide delivery, which has become a reality.[44,51] Moreover, future work is needed to explore the therapeutic potential of combination therapy in augmenting the endogenous NP system and to identify the optimal strategies for incorporating biomarkers to guide therapy.

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