Potential of Natriuretic Peptides as Therapeutic Agents in Cardiovascular Disease

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1. Introduction

Congestive heart failure (CHF) and hypertension are major clinical entities with an extraordinary impact in our society given their morbidity and mortality. Treatment of these conditions involves the administration of polypharmacy using pharmacological agents such as diuretics, angiotensin converting enzyme inhibitors, angiotensin receptor blockers, beta blockers, calcium channel antagonists, etc. Although these are effective they are not without side-effects and many are extremely expensive while mortality and morbidity has not decreased.

Most of the therapeutic effects of the above agents are combined in the natriuretic peptides ANF and BNP. ANF, BNP and CNP constitute a family of peptide hormones sharing sequence, structural and functional similarities (19). These peptides are encoded by unique genes. The biologically active species are cleaved from the carboxyl termini of their respective precursor molecules (1; 14; 19). ANF and BNP are synthesized in the heart in a regulated manner by atrial cardiocytes. CNP is mainly produced in a constitutive fashion in vascular endothelial cells and in the brain and is not diuretic/natriuretic nor does it have effects on the renin-angiotensin-aldosterone system (14).

ANF and BNP exhibit natriuretic and vasorelaxant properties generally opposing the renin-angiotensin-aldosterone system and the sympathetic nervous system (20; 29; 37). These biological effects are exerted through specific membrane-bound guanylyl cyclase receptors, NPR-A (ANF and BNP) and NPR-B (CNP), thereby increasing intracellular 3',5-cyclic guanosine monophosphate (cGMP) (1; 42). cGMP plasma levels and urinary excretion increase in parallel to increases in ANF and BNP plasma concentrations and hence, determination of cGMP in plasma or its excretion in urine can be used as a reflection of ANF or BNP plasma levels. BNP is about 10 fold less potent than ANF in promoting cGMP production (1; 42) A third receptor, NPR-C, is a clearance receptor (14; 42). Neutral endopeptidases, present in the kidney in vascular and tubular cells also degrade ANF, BNP and CNP (42).

ANF prevents hypertrophy of adult rat cardiocytes by a process that involves the activation of NPR-A (28). Genetic disruption of the NPR-A gene induces salt resistance hypertension, cardiac hypertrophy and fibrosis. In contrast, BNP knockouts do not develop hypertension or cardiac hypertrophy but show cardiac fibrosis (14; 42).

Comparative binding studies to NPR-A in vascular smooth muscle between ANF and BNP showed a Kd of 1.6 pM for ANF and 7.3 pM for BNP suggesting that the potency for vasorelaxation is markedly less for BNP compared to ANF due to the lower affinity of BNP for binding to the NPR-A receptor (14).

The treatment of heart failure with ANF or BNP has been carried out with excellent results (2; 11; 12; 37). However, the peptide nature of these hormones prevents their administration by ingestion and even when injected, they typically show very short (~2 min) half-lives in blood due to its rapid clearance. The need for continuous intravenous (iv) infusion limits its use to a hospital setting. Therefore, the development of an ANF analog with significantly longer half life in blood appears a worthwhile pursuit.

A number of studies have assessed the efficacy of ANF infusion in CHF in humans and animals (2; 3; 17; 25; 29; 37; 41).

Most studies using ANF in humans have been carried out in a hospital setting using iv administration of synthetic human (h) ANF. An iv injection of hANF to patients with mild hypertension or with CHF results in a decrease in systolic blood pressure, pre-load, after-load, renin activity and improvement in left ventricular performance without adverse side-effects (7; 21; 26; 30; 40). Long term iv administration of ANF (Carperitide®) for 7 days in patients with acute CHF “who resisted various therapies” showed a significant hemodynamic improvement 48 h after the start of the infusion. These patients showed a significant decrease in mean pulmonary wedge pressure, mean right atrial pressure, systemic vascular resistance but no changes in systolic blood pressure or heart rate (5). Hayashi et al. (8) showed that a 24 h infusion of synthetic ANF (0.025 µg/kg/min) in patients with first anterior acute myocardial infarction (AMI) prevented left ventricular remodeling and improved left ventricular ejection fraction. In addition, plasma levels of aldosterone, endothelin (ET-1) and angiotensin (Ang) II were significantly decreased during the ANF infusion. It was suggested that the beneficial effects of ANF administration in these patients may be partly due to the suppression of aldosterone, ET-1 and Ang II secretion.

Beneficial effects of iv ANF administration after AMI were recently reported by Kuga et al., in a small group of patients (13). The patients received an intracoronary bolus of ANF (25 µg) within 12 h after AMI and an iv of 0.0025 µg/kg/min initiated on admission and maintained for one week. A similar group of patients received saline. ANF-treated patients showed a significant increase in left ventricular ejection fraction. Left ventricular end-diastolic volume index decreased significantly at six months as compared to saline-treated patients. Left ventricular regional wall motion of the infarcted segments also increased significantly in the ANF group. These results suggest that administration of ANF prevented reperfusion injury to the myocardium and conserved LV function by improvement of regional wall motion of the infarcted segments. Finally, chronic infusion (>48 h) of ANF (50 µg/kg/min) was shown to improve renal blood flow and glomerular filtration rate in patients with acute renal impairment associated with cardiac surgery (38).

The above results show that ANF administration increase cGMP generation, decrease cardiac preload and afterload and improve left ventricular systolic and diastolic functions in hypertension and CHF, but as a treatment it is restricted to continuous intravenous use.

Recently, the safety, efficacy, and therapeutic benefits of Natrecor® (synthetic BNP) have been demonstrated by the VMAC and PRESEDENT clinical studies (6; 9; 10). BNP use, however, is limited to decompensated acute heart failure in a hospital setting due to the need for intravenous infusion.

Long term increases on circulating ANF appears to be a desirable therapeutic approach in the treatment of hypertension, AMI and CHF. However, the peptide nature of ANF necessitates its intravenous administration and its relative short half life in circulation, severely complicates the logistics of using natriuretic peptides as a therapeutic agent. Therefore, the development of ANF analogs that would be active using an alternative route of administration and for which half-life is increased appears as a worthwhile pursuit.

It is possible to obtain more stable forms of a circulating protein using gene fusion technology utilizing the coding sequences for the protein or peptide of interest and human serum albumin (HSA). The conjugate thus obtained is expected to be, like HSA, slowly cleared by the liver, have an *in vivo* half life of ~ 19 days and be devoid of any enzymatic activity or immunogenicity. These particular features have been demonstrated previously (4; 15; 22-24; 43). Proteins resulting from HSA gene fusions showing a relatively long half lives while retaining their biological and therapeutic properties have been successfully produced (16; 18; 31-36; 39).

We have previously shown that 2-HSA (15), an ANF derivative that chemically cross-links to human serum albumin (HSA), administered subcutaneously (sc), produced biological responses similar to that of native ANF but had significantly longer lasting effects (72 h) on cardiovascular and renal function in spontaneously hypertensive (SHR) and Wistar Kyoto (WKY) rats when compared to native ANF thus providing proof of principle.

There are several problems related to the production of compounds such as the ANF derivative mentioned above by chemical means. Namely, it is possible that chemical cross-linking to serum albumins could generate errors innate to the cross-linking procedure, as has been shown by Poznansky et al (27) raising important limitations concerning the exact formulation and reliability of such pharmaceutical preparations, including immunogenicity. In addition, the cost of production is commercially prohibitive. These limitations can be avoided by using genetic engineering techniques to produce an ANF-HSA fusion protein that can be expressed in a scalable cell system and easily recovered in a homogenous state. The use of ANF appears more advantageous than the use of BNP mainly because ANF has a higher affinity for NPR-A. In addition, the amino acid sequence of ANF is highly conserved, which facilitates the use of non-human test systems (14).

In conclusion, ANF expressed together with a protein such as HSA has the potential to become a useful compound for the treatment of CHF and myocardial remodeling following acute myocardial infarction.

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