

Frontiers in cardiovascular medicine

Neutral endopeptidase inhibition and the natriuretic peptide system: an evolving strategy in cardiovascular therapeutics

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Received 29 May 2012; revised 20 July 2012; accepted 28 July 2012; online publish-ahead-of-print 31 August 2012

Hypertension and heart failure (HF) are common diseases that, despite advances in medical therapy, continue to be associated with high morbidity and mortality. Therefore, innovative therapeutic strategies are needed. Inhibition of the neutral endopeptidase (NEP_{inh}) has been investigated as a potential novel therapeutic approach because of its ability to increase the plasma concentrations of the natriuretic peptides (NPs). Indeed, the NPs have potent natriuretic and vasodilator properties, inhibit the activity of the renin–angiotensin–aldosterone system, lower sympathetic drive, and have antiproliferative and antihypertrophic effects. Such potentially beneficial effects can be theoretically achieved by the use of NEP_{inh}. However, studies have shown that NEP_{inh} alone does not result in clinically meaningful blood pressure-lowering actions. More recently, NEP_{inh} has been used in combination with other cardiovascular agents, such as angiotensin-converting enzyme inhibitors, and antagonists of the angiotensin receptor. Another future possible combination would be the use of NEP_{inh} with NPs or their newly developed chimeric peptides. This review summarizes the current knowledge of the use and effects of NEP_{inh} alone or in combination with other therapeutic agents for the treatment of human cardiovascular disease such as HF and hypertension.

Keywords Neutral endopeptidase inhibition • Natriuretic peptide system • Evolving strategy in cardiovascular therapeutics

Introduction

The burden of cardiovascular disease (CVD) continues to increase worldwide. The final common pathway in CVD is heart failure (HF), which often is mediated by progressive uncontrolled hypertension. Indeed, the important link between hypertension and HF is underscored by the recent report from the landmark ALLHAT Study that demonstrated that the development of HF in hypertensive patients was a powerful predictor for increased mortality¹. A recent report further suggested the importance of the control of hypertension for the reduction in HF.²

Today, there continues to be a high priority for the development of innovative therapeutic agents that better control blood pressure (BP) and also have a therapeutic potential in the setting of HF. Such agents should enhance current therapies for CVD and, importantly, prevent target-organ damage. Such therapeutics could be

especially useful for high-risk populations such as the elderly, diabetics, African-Americans, and other populations in whom adequate BP control is of great importance. Currently, there is a widespread use of modulators of the renin–angiotensin–aldosterone system (RAAS) which may inhibit release of renin, antagonize angiotensin II (Ang II) at its receptor level, or block the actions of aldosterone. Such strategies underscore the deleterious properties of over-activated RAAS, which is a hallmark of CVD.

In this review, we will focus on an endogenous peptide system, the natriuretic peptide (NP) system, and on novel strategies aimed to enhance the biological activities of the NPs via inhibition of their degradation.

As will be discussed below, manipulation of this system so as to achieve target-organ protection, BP control, optimal volume homeostasis, and the inhibition or reversal of myocardial and renal remodeling represents a new therapeutic opportunity.

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Our focus will be upon inhibition of a key enzyme which degrades the NPs, specifically, neprilysin (neutral endopeptidase or EC 3.4.24.11 or NEP), and on the combination of NEP inhibition and RAAS antagonism by novel molecules.

Natriuretic peptide system

The NP system consists primarily of three well-characterized peptides, with each being a distinct gene product with structural similarity (Figure 1): atrial natriuretic peptide (ANP)³ and B-type natriuretic peptide (BNP)⁴ are mainly from cardiomyocytes, and C-type natriuretic peptide (CNP) is mostly from endothelial and renal cells.^{5–8} These three peptides all have cardiorenal protective properties. Of note, BNP is also produced by cardiofibroblasts where it elicits its anti-fibrotic actions in the heart.⁹ While ANP is secreted from the myocytes as the active hormone ANP1–28, BNP is produced and released from the myocytes as a 108 amino acid prohormone, proBNP1–108, in a glycosylated form.¹⁰ In the circulation, glycosylated proBNP1–108 is gradually deglycosylated and further processed by corin into the biologically active BNP1–32 and into the inactive N-terminal (NT)-proBNP1–76

linear fragment.¹¹ Like ANP and BNP, CNP is formed by cleavage of a precursor protein, proCNP1–103, by proteolytic enzymes to produce the active forms CNP1–22 and CNP1–53.¹²

While ANP and BNP are released from the myocytes in response to cardiac stretch, CNP is released from endothelial cells in response to cytokines and endothelium-dependent agonists, such as acetylcholine. Like ANP and BNP, CNP has potent systemic cardiovascular actions, which include reductions in cardiac filling pressures and output, secondary to vasorelaxation and decreases in venous return, but has minimal renal actions.¹³ CNP is the most anti-fibrotic of the three native endogenous NPs.

As illustrated in Figure 2, all NPs function via the second messenger cGMP. Atrial natriuretic peptide and BNP bind to GC-A while CNP binds to the GC-B¹⁴ (Figure 2). All three peptides are cleared by the clearance receptor, NPR-C, which is a receptor that is not linked to a GC.¹⁵ New evidence, however, indicates that the NPR-C is also involved in the anti-fibrotic actions of the NP by mechanisms that are independent of cGMP activation.¹⁶ The NPs are also cleared from the circulation via enzymatic degradation by NEP.¹⁴ A comprehensive view of the biology of these peptides has emerged following studies in cell systems, murine models of

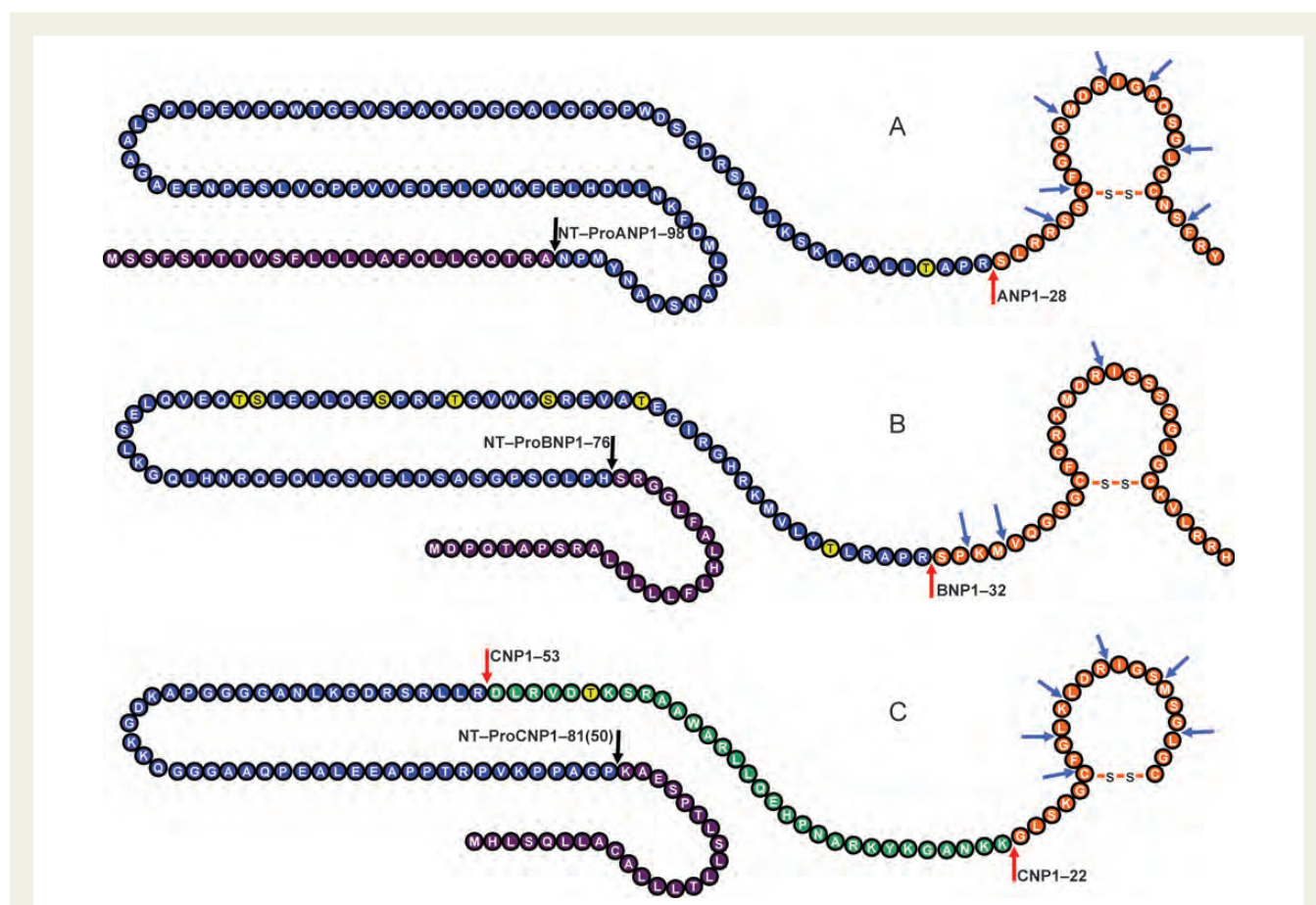


Figure 1 The three major human endogenous natriuretic peptides, their processing and degradation. (A) ProANP1–126 and signal peptide, cleaved to NT-ProANP1–98 and the active hormone ANP1–28. (B) ProBNP1–108 and signal peptide, cleaved to NT-ProBNP1–76 and the active hormone BNP1–32. (C) ProCNP1–103 and signal peptide, cleaved to NT-ProCNP1–80(50) and the active hormones CNP1–22 and CNP1–53. Red arrows indicate processing site by corin or furin. Blue arrows indicate neutral endopeptidase cleavage sites. Yellow-labelled amino acids indicate glycosylation sites that may prevent processing. Purple-labelled amino acid sequence indicates the signal peptide.

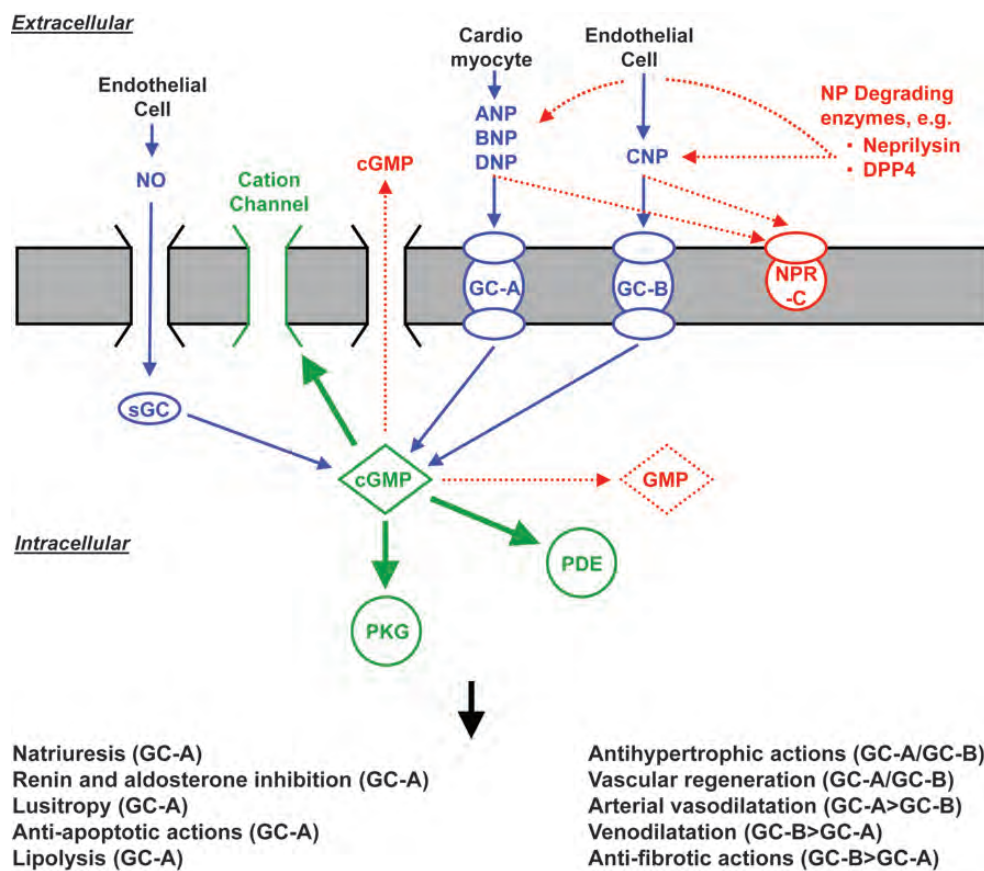


Figure 2 Known actions of the natriuretic peptides through GC-A and GC-B activation. ANP, atrial natriuretic peptide; BNP, B-type natriuretic peptide; CNP, C-type natriuretic peptide; DNP, dendroaspis natriuretic peptide; cGMP, cyclic guanosine monophosphate; sGC, soluble guanylyl cyclase; GC-A, guanylyl cyclase-A; GC-B, guanylyl cyclase-B; NO, nitric oxide; NPR-C, natriuretic peptide receptor-C; PDE, phosphodiesterase; PKG, protein kinase G; DPP4, dipeptidyl peptidase IV.

altered NP production or receptor function, and integrative physiological studies in disease models and in humans. The biological properties of these peptides, which include natriuresis, vasodilatation, inhibition of the RAAS, positive lusitropism, and inhibition of fibrosis, have led to the unique concept of cardiorenal protection by activation of cGMP.^{15,17–21} Such a view has been strengthened most recently by successful chronic therapeutic strategies in experimental CVD states. Specifically, oral delivery of BNP with novel conjugation technologies has been reported in dogs.²² Most exciting is the recent success of direct cardiac BNP overexpression achieved with a novel gene delivery system in rats via an adeno-associated virus (AAV9) vector. Indeed, this approach allows long-term cardiac delivery of BNP for the treatment of hypertensive heart disease and induces improvement of cardiac function and structure.²³

Most recently, there has been a flurry of new data demonstrating the actions of the NPs, particularly the GC-A agonists ANP and BNP, on metabolic regulation. Among the many potentially beneficial findings are that GC-A activation increases lipid oxidation in transgenic rodents, inhibits adipocyte growth, increases oxygen consumption, increases mitochondrial biogenesis in the skeletal

muscle in rodents, delays gastric emptying, activates adiponectin, converts white adipocytes to brown adipocytes, lowers insulin levels, and improves glucose tolerance.^{24–32} Furthermore, a specific human ANP genetic variant rs5068, which increases circulating ANP, protects against both hypertension and metabolic syndrome.^{33,34} Of note, hypertension and obesity are both associated with reduced ANP and BNP levels, suggesting the existence of a deficiency of the NP system in these conditions thus supporting a need for NP therapy.^{35–38}

Although initially studied for their diagnostic and predictive value in human HF, the NPs are now also viewed as potential, innovative therapeutic agents for cardiorenal and, perhaps, even in metabolic disease syndromes. Indeed, a seminal work published back in 1986 in *Science*, marked the beginning of the use of the NPs as markers of human CVD.³⁹ Since then, the NPs and, in particular, the BNP1–32 and NT-proBNP 1–76 molecular forms have been used for the diagnosis and prognosis of HF. However, due to the inherent biological properties of the NPs, these hormones are now considered as useful treatment of HF and other CV morbidities such as hypertension.^{40–43} To date, ANP and BNP are currently approved for the treatment of acute HF in Japan and in

USA, respectively. While the ASCEND-HF trial could not demonstrate reduced rate of death or rehospitalization or symptom relief with Nesiritide (recombinant BNP), it confirmed the safety of the drug regarding renal function.⁴⁴ The higher occurrence of hypotension after Nesiritide in this trial may suggest that this type of treatment could be dose adjusted so as to avoid excessive hypotension and could be targeted to benefit certain subgroups within the acute HF population.⁴⁴ Most recently, Cataliotti *et al.*⁴⁵ have demonstrated the efficacy of small dose (10 µg/kg twice a day) of subcutaneously delivered BNP in normalizing BP in a patient with uncontrolled hypertension even when the subject's usual antihypertensive therapy was not given. Indeed, this is the first evidence of the efficacy of these peptides in human hypertension and may represent a new concept in the treatment of resistant hypertension.

Most recently, a designer NP, Cenderitide or CD-NP, has entered clinical trials for HF.^{46,47} Cenderitide, is a newly designed chimeric peptide that simultaneously co-activates GC-A and the GC-B by fusing together the amino acid sequence of CNP, a pure GC-B agonist, with the elongated C-terminal tail sequence of a GC-A agonist found in snake venom, dendroaspis NP.⁴⁸ Neutel *et al.*⁴⁹ recently reported the results of a Phase I clinical trial evaluating the pharmacokinetics, pharmacodynamics, safety, and tolerability of subcutaneous bolus and subcutaneous infusion of Cenderitide in patients with chronic HF. Cenderitide was well tolerated and, compared with native NPs, possesses greater resistance to degradation by NEP.⁵⁰ Given the encouraging results obtained with the use of the native NPs and their chimerics, like Cenderitide, in the treatment of human CVD and yet the difficulties associated with the chronic delivery of proteins such as the NPs, the prevention of their enzymatic degradation by NEP, so as to enhance the NP system, is a target worth pursuing. Thus, the effects of NEP inhibition (NEP_{inh}) have been explored both in animal models and in humans either alone or in combination with the inhibition of other systems involved in the progression of CVD.

Dysregulation of the natriuretic peptide system in hypertension and heart failure

There is a growing body of evidence that dysregulation of the NP system exists in human CVD. As stated above, human hypertension, HF, and obesity may all be an NP deficiency state.^{36–38} Initially, it was thought that during the evolution of HF, the heart increases its production and release of ANP and BNP, in order to compensate for the increased water retention and cardiac stress that characterizes left ventricular (LV) systolic dysfunction.³⁹ Indeed, elevated circulating levels of such hormones, as detected by commercially available immunoreactive assays, have been associated with worsening of HF and with poor prognosis.⁵¹ Recent studies using more sophisticated technologies (sensitive mass spectrometric techniques) have reported that in patients with congestive HF and high plasma BNP levels assessed by a conventional diagnostic assay (Biosite), there is actually a lack of mature BNP1–32.⁵² Thus, this suggested that altered processing of proBNP1–108 and/or of BNP1–32 might occur in HF and

hence a relative deficiency of this protective hormone. Studies have now confirmed our previous report and support the concept that altered processing of BNP does indeed occur in HF. Niederkofer *et al.*,⁵³ using an extremely sensitive mass spectrometry-based method, have recently reported very low levels of circulating BNP1–32 in subjects with advanced HF despite markedly elevated levels of immunoreactive BNP by the Biosite assay. Furthermore, they also demonstrated the presence of shorter, less biologically active, proteolytically processed forms of BNP1–32. These results were further confirmed in a later study by Miller *et al.*⁵⁴ Here, the authors also used a quantitative mass spectrometry approach to determine the circulating molecular forms of BNP in patients with HF. They demonstrated that less biologically active precursors and multiple degraded forms of BNP are present and are included in the measurements that come from the current clinical assays. The authors suggested that most of the 'BNP' that is detected by clinical BNP assays is actually different degradation products of BNP and the less biologically active prohormone, proBNP1–108. Taken together, these data help explain the blunting of the expected physiological responses to apparently high levels of BNP. Furthermore, it suggests that patients with advanced HF may actually be in a state of NP deficiency and may benefit from exogenous administration of recombinant NP or by the inhibition of NP proteolytic degradation.

Similar to what was observed in HF, patients with essential hypertension may also have an NP deficiency state. Although initially thought that this hormone was activated in hypertension, Belluaro *et al.*³⁷ reported in 2006 the presence of a relative deficiency of the BNP system in early stages of essential hypertension. In particular, BNP1–32 was not activated in hypertensive patients, while NT-proBNP was lower in mildly hypertensive patients when compared with normotensive subjects. This suggested that a deficiency of bioactive BNP may be present in the early stages of hypertension thus favoring the progression of this condition. Importantly, we have now extended this original observation to a large well-characterized adult general population from Olmsted County. Here, we also defined the ANP system to investigate whether the deficiency of the BNP system in subjects with mild hypertension is counterbalanced by the activation of the other cardiac hormone. However, we not only confirmed that the BNP system including BNP1–32, NT-proBNP, and the precursor proBNP1–108 was not activated, but also demonstrated that the ANP system, by ANP1–28 and NT-proANP1–98, was likewise lower in pre-hypertensives than in normotensive subjects.³⁸

Dries *et al.*⁵⁵ have also reported that the presence of two missense mutations in the corin gene (the enzyme that is known to process proBNP1–108 to its active form BNP1–32 as well as non-active proANP1–126 to mature ANP1–28) is associated with high BP and hypertension in African-Americans. This and similar findings reported in a mouse model, in which the deletion of corin induced hypertension, support the hypothesis that an altered processing pathway of proBNP1–108 to mature BNP1–32 also occurs in hypertension.⁵⁶ Perhaps, this impaired processing of the NP results in reduced BP-lowering effects of these vasoactive hormones and so leads to an increased risk of developing hypertension and ultimately to more severe CVD such as overt HF. Complementing the elegant work of Dries are the studies of Newton-Cheh *et al.*

Here, these investigators employing large populations have identified single-nucleotide polymorphisms of the ANP and BNP gene as well as the NP clearance receptor, which are linked to a protection from hypertension when associated with elevated circulating NP levels.^{33,57} Taken all together, these studies support the existence of a deficiency state of biologically active cardiac NPs in HF and hypertension. The use of these hormones or of strategies aimed at preventing their excessive degradation for the treatment of CVD is therefore a logical pursuit.

Neutral endopeptidase

Neutral endopeptidase is a type II integral membrane metallopeptidase. Specifically, it is a zinc-dependent, membrane bound endopeptidase that hydrolyses peptides on the amino side of hydrophobic residues.^{58–61} Neutral endopeptidase has a short NT cytoplasmic domain, a single transmembrane helix, and a C-terminal extracellular domain with a zinc atom at the active site.^{62,63} In mammals, NEP is widely expressed, e.g. kidney, lung, endothelial cells, vascular smooth muscle cells, cardiac myocytes, fibroblasts, neutrophils, adipocytes, testes, and brain, with the highest concentrations being present in the renal proximal tubule. In lymphocytes, NEP expression is developmentally regulated.^{61,64–67} Neutral endopeptidase is critical for the processing and catabolism of vasoactive peptides and peptides involved in diuresis and natriuresis, e.g. the NPs, angiotensin I (Ang I), bradykinin (BK), and endothelin-1 (ET-1).^{68–70} Many other substrates for NEP exist, including opioid peptides, Substance P, peptides involved in the regulation of inflammation, amyloid β -protein, and gastrin. Neutral endopeptidase's ability to degrade multiple substrates also means that the sole inhibition of NEP yields broader effects than anticipated and explains why NEPinh is best combined with the inhibition of other vasoactive compounds.

Selective neutral endopeptidase inhibition

Medical therapy for hypertension and HF is usually aimed at decreasing cardiac load by haemodynamic modulation. Typical ways of accomplishing this are by arterial dilatation, venous dilatation, and increased sodium and water excretion. Indeed, angiotensin-converting enzyme (ACE)-inhibitors, which block the conversion of Ang I to Ang II, suppress the RAAS, and increase BK levels, are known to decrease cardiac afterload and reduce HF morbidity and mortality.^{71,72}

Since many substrates for NEP are peptides with vasoactive and diuretic/natriuretic actions; hence, NEPinh has been examined as a potential therapeutic modality. The key role that NEP plays in the degradation of the NPs initially provided the rationale for NEPinh. Atrial natriuretic peptide is cleaved by NEP at seven different sites, but the initial cleavage occurs between Cys7 and Phe8. Cleavage here destroys ANP's ring structure and so inactivates it.⁶⁸ Likewise, CNP is cleaved between Cys7 and Phe8 and between other hydrophobic amino acids.^{73,74} B-type natriuretic peptide, although hydrolysed by NEP, is slightly more resistant to NEP than ANP or CNP. Neutral endopeptidase cleaves BNP first between Met4 and Val5 and then at other sites.^{75,76}

If NEP solely acted on NPs, NEPinh alone might be expected to augment the vasodilating, natriuretic, and diuretic actions seen with NPs. However, NEP hydrolyses other vasoactive peptides with opposing physiological actions.⁷⁷ Thus, NEPinh alone results in both desirable and undesirable effects. For example, NEP hydrolyses Ang I to angiotensin 1–7, and since Ang 1–7 counters the action of the vasoconstrictor Ang II, the hydrolysis of Ang I to Ang 1–7 by NEP produces a potentially beneficial BP-lowering effect. Also desirable, NEP catabolizes the potent vasoconstrictor ET-1. However, NEP also hydrolyses BK to the inactivated BK 1–7, which results in the undesirable effect of BP increasing. In short, NEPinh would help increase the circulating levels of the NPs and BK, but it would also increase the vasoconstrictors Ang II and ET-1. Some studies have even reported greater vasoconstrictor than vasodilator effects from NEPinh alone.⁷⁷

One of the first NEP inhibitor developed for clinical use was candoxatril, a potent, orally available NEP inhibitor that is, however, no longer clinically studied (Candoxatrilat was a related, intravenously administered compound). In humans candoxatril caused a dose-dependent increase in plasma ANP, natriuresis, and the NP second messenger, cGMP.^{78,79} However, NEPinh with candoxatril was also found to increase Ang II levels. This Ang II increase was not accompanied by elevated aldosterone levels, most likely secondary to the concomitant increase in NPs, which are known to suppress aldosterone.^{80–83} Ultimately, candoxatril's effects on BP in hypertensive patients were not consistently impressive. Higher doses (200 mg) were more natriuretic than lower doses.⁸⁴ When candoxatril 200 mg, twice daily for 28 days, was compared with placebo in essential hypertension patients, no relevant BP decrease occurred despite significantly increased ANP levels.⁸⁵ Taken together, NEPinh in hypertensive subjects produces the competing effects of increased pressors and increased vasodilators, with an insignificant BP-lowering result.

Candoxatril was also studied in the setting of chronic LV dysfunction, and this has been extensively reviewed elsewhere.⁷⁹ In LV dysfunction, vasoconstrictor and vasodilator balance is paramount. In a canine model of mild HF (characterized by elevations of NPs but not RAAS), candoxatril improved renal haemodynamics and increased urinary ANP and cGMP excretion. These beneficial actions were not found when a severe HF model (characterized by both NP elevation and RAAS activation) was similarly treated with candoxatril.⁸⁶ In human HF, candoxatril or candoxatrilat treatment increased ANP and BNP levels, produced diuresis and natriuresis, and decreased clearance of ANP administered exogenously. However, systemic and pulmonary vascular resistances were not affected.⁷⁹ In small studies of chronic HF patients, candoxatril increased levels of the vasoconstrictor ET-1 as well as ANP levels⁸⁷ and also caused a dose-dependent increase in systemic vascular resistance and decrease in cardiac index.⁸⁸

Dual inhibition of neutral endopeptidase and angiotensin-converting enzyme

Given that NEPinh alone leads to an increase in circulating levels of both vasodilators as well as vasoconstrictors, drugs that inhibited

both NEP and ACE were developed and are referred to as vaso-peptidase inhibitors (VPIs).⁸⁹ The appeal of this combination is that NEP inhibition increases endogenous NP levels and that ACE inhibition attenuates the Ang II increases seen with NEP inhibition alone. Thus, the combination should decrease systemic and renal vascular resistance, suppress aldosterone, and increase natriuresis and diuresis.⁹⁰ Currently, however, none of these compounds are in clinical development. In preclinical studies, these compounds showed promise for hypertension, HF, and renal disease treatment; however, their side effect profile in clinical trials stunted development. Of particular concern was the high incidence of angioedema, which is typically manifested as swelling of the skin and mucous membranes of the face, lips, tongue, oropharynx, upper respiratory tract, and occasionally intestines.⁹¹

Omapatrilat was the VPI in the most advanced stage of development. It was compared with lisinopril in HF patients in the IMPRESS trial. It performed well in the composite of death, admission, or study treatment discontinuation for worsening HF, and it improved NYHA class more than lisinopril.⁹² After these promising results, the OVERTURE trial compared omapatrilat with enalapril in chronic HF patients. Unfortunately, here the investigators did not

find superiority of omapatrilat over enalapril with respect to the primary endpoint, the combined risk of death or hospitalization for HF requiring intravenous treatment.⁹³ Eventually, a Food and Drug Administration (FDA) review of an omapatrilat safety database found a relatively high occurrence (compared with ACE inhibition alone) of severe angioedema, especially in African-Americans and smokers, and did not approve the drug.⁹⁴ The OCTAVE trial was designed as a large ($n = 25\,302$), randomized, active-controlled, multicentred trial that compared 6 months of treatment with omapatrilat or enalapril in hypertensive patients.⁹⁵ Patients were started at a low dose of omapatrilat and titrated up in the hope of decreasing the incidence of angioedema. By Week 8, omapatrilat reduced systolic BP 3.6 mmHg more than enalapril. By Week 24, omapatrilat-treated subjects required less adjunctive antihypertensive therapy than enalapril-treated patients. Omapatrilat-treated subjects were more likely to reach goal BP, but again they had a higher incidence of angioedema (2.17% for omapatrilat vs. 0.68% for enalapril), which occurred early in the course of therapy.⁹⁵ Ultimately, omapatrilat was not FDA approved.

Angioedema development with VPIs remains a persistent concern. It had been simply thought that increased levels of BK

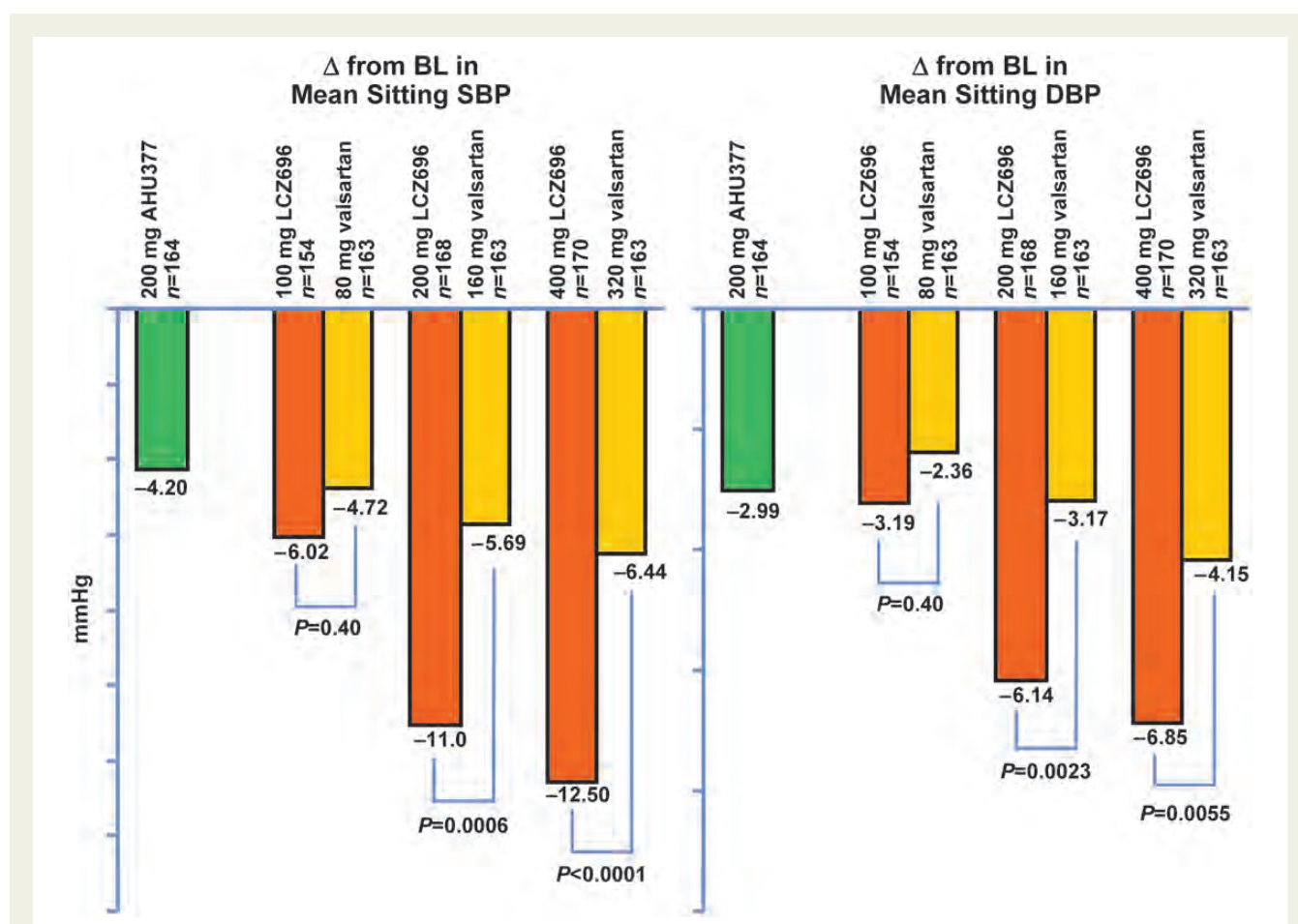


Figure 3 Angiotensin receptor blockade with neutral endopeptidase inhibition effects: decreased blood pressure as a result of the direct antihypertrophic, anti-fibrotic, endothelial, and renal-enhancing actions mediated by increased biological activities of the natriuretic peptides and by increased angiotensin type 2 receptor binding (adapted from Ruilope et al.,¹⁰¹ Copyright 2010, with permission from Elsevier).

secondary to dual ACE and NEP inhibition were the cause of the angioedema. We now know that inhibition of aminopeptidase P (APP), which was also potently inhibited by omapatrilat, is an important consideration.⁹⁶ Aminopeptidase P has a role both in BK degradation when ACE is inhibited and in the inactivation of the pro-inflammatory BK metabolite, des-Arg⁹-BK. Indeed, subjects with lower APP levels are predisposed to develop angioedema with ACE-inhibitor therapy.⁹⁷ Similarly, individuals with XPNPEP2 genetic variations that result in lower APP levels are also more apt to have ACE-inhibitor-associated angioedema.^{98,99} In this age of pharmacogenomics and personalized therapy, the incidence of angioedema may be decreased by not giving genetically predisposed subjects such a medication.

Angiotensin receptor blockade with neutral endopeptidase inhibition

An alternative dual NEP and ACE inhibition strategy can be achieved by the combination of NEPinh with an angiotensin receptor blocker (ARB). Angiotensin receptor blockers do not disrupt BK metabolism as much as ACE-inhibitors, and it has been reported that some patients with ACE-inhibitor-associated angioedema can be switched over to an ARB without the occurrence of angioedema.⁹⁷ This has led to the development of a novel class of drugs that combines the actions of NEPinh and ARB, known as angiotensin receptor blockade with neutral endopeptidase inhibition (ARNi).

LCZ696 is the first compound of this category and the one in the most advanced stage of development. This ARNi is orally available and provides in a 1:1 ratio blockade of the angiotensin type 1 receptor (AT1R) with a valsartan moiety and NEPinh via an AHU377 prodrug moiety, which is metabolized within an hour to the active moiety LBQ657.¹⁰⁰ In an 8-week, randomized, double-blind Phase II trial in hypertensive patients, various doses of LCZ696 were compared with those of comparable doses of valsartan. ARNi-treated subjects had greater reductions in sitting systolic and diastolic BP than valsartan-treated subjects. This was significant for the group treated with 200 mg LCZ696, ('mid range' dose) vs. 160 mg valsartan and for 400 mg LCZ696 (highest dose) vs. 320 mg valsartan. The ARNi-treated subjects also had greater systolic and diastolic BP reductions than found in a separate group treated with AHU377 alone (Figure 3). Angioedema did not occur, with ~8% of the ARNi-treated subjects being black.¹⁰¹

There are currently ongoing trials examining LCZ696 both in chronic HF and in chronic HF with preserved ejection fraction (EF), and studies evaluating sodium excretion in healthy, hypertensive, and HF patients treated with LCZ696. Of great interest, the outcome of the PARADIGM-HF trial is eagerly awaited. In this Phase III study in symptomatic HF, the ability of LCZ696 to delay the first occurrence of HF hospitalization or CV mortality will be compared with enalapril.¹⁰² Paramount HF will test the hypothesis that in patients with HF and preserved EF, LCZ696 will have favorable actions on neurohormones and on echocardiographic findings with chronic therapy compared with ACE inhibition.¹⁰³

Table 1 Main actions of angiotensin type 1 and 2 receptor activation

Receptors	Local actions		Clinical effects			
	AT1R	AT2R	Hypertrophy	Antihypertrophy	Proliferation	Anti-proliferation
			Vasoconstriction	Vasodilatation		
					Pro-fibrotic	Anti-fibrotic
					Pro-oxidation	Anti-oxidation
			Hypertension	Hypotension	Cardiac and vascular remodelling	Anti-cardiac and -vascular remodelling
						Atherosclerosis
						Anti-atherosclerosis

To complement LCZ696, other innovative ARNi's are being developed. Specifically, a highly potent, oral, dual inhibitor of the AT1R and neprilysin for the treatment of hypertension and/or chronic HF is under development. This newest ARNi, still in pre-clinical development, is a single molecule exhibiting pharmacological properties of both NEPinh and AT1R blockade.¹⁰⁴

Summary

Using NEPinh to increase the circulating plasma concentrations of NPs in disease states such as HF and hypertension, which are marked by relative deficiencies of bioactive NPs, set the way for VPIs and ARNi's. Unfortunately, these early VPIs, in addition to favourable therapeutic actions, were also associated with an increased risk of angioedema. This side effect caused the FDA not to approve the most clinically advanced VPI, omapatrilat.

ARNi's represent the latest advance combining an ARB with NEPinh, which are now being evaluated in preclinical and clinical studies. Indeed, the combination of an NEP inhibitor and ARB is very appealing. Blockade of the AT1R by ARB facilitates the binding of Ang II to the angiotensin type 2 receptor that elicits several favorable actions (Table 1). LCZ696 stands out as one of the first drugs of this ARNi class that combines inhibition of NEP and blockade of the AT1R. Importantly, this novel class of drugs seems to be safe and not to share with their VPIs precursors an increased risk for angioedema neither in more at-risk subjects such as African-Americans. The dual inhibition of the Ang II receptor and NEP could provide clinical benefits in a wide range of CVDs, particularly hypertension and HF. We eagerly await the result of clinical trials examining ARNi both in chronic HF and in chronic HF with preserved EF.

Funding

Supported by grants from the National Institutes of Health: HL R01 36634, HL P01 76611, HL R01 098502-01A1, and the Mayo Foundation, Rochester, MN, USA.

Conflict of interest: Mayo Clinic has licensed Cenderitide (CD-NP) to Nile Therapeutics and J.C.B. is Chair of the Scientific Advisory Board of Nile Therapeutics.

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