Neprilysin Inhibition in Chronic Kidney Disease

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ABSTRACT (200 words)

Purpose of review:

Chronic kidney disease (CKD) is associated with increased risk of progression to end-stage kidney disease and cardiovascular events. There is limited evidence that available treatments have beneficial effects on cardiorenal outcomes in all people with non-diabetic CKD. Neprilysin inhibition (NEPi) is a new therapeutic strategy with potential to improve outcomes for patients with CKD.

Recent findings:

NEPi enhances the activity of the natriuretic peptide system producing natriuresis, diuresis and inhibition of the renin-angiotensin system (RAS) and sympathetic nervous system. Sacubitril/valsartan is the first angiotensin receptor neprilysin inhibitors (ARNi) to be produced and has been shown to substantially improve cardiovascular outcomes in heart failure and delay progression of kidney disease in this population. Although ARNIs have not shown similar effects on kidney function in the short-to-medium term in people with CKD, they are associated with substantial reductions in cardiac biomarkers and blood pressure in CKD.

Summary:

These data suggest that NEPi with an ARNI could benefit patients with CKD by reducing the risk of cardiovascular disease and have the possibility of retarding the progression of CKD (hence delaying the need for renal replacement therapy).

Keywords:

Neprilysin, cardiovascular disease, kidney disease

Introduction

Chronic kidney disease (CKD) is associated with two major hazards: increased risk of progression to end-stage kidney disease (ESKD) requiring treatment with renal replacement therapy (dialysis or transplantation) and premature morbidity and mortality from cardiovascular disease (CVD).[1-3] Randomized trials have demonstrated renin-angiotensin system (RAS) inhibitors slow the progression of diabetic and non-diabetic proteinuric CKD.[4-7] However, despite these treatments, significant risk of progression to ESKD and cardiovascular events remains.

As CKD progresses, the manifestation of CVD changes from atherosclerotic disease to non-atherosclerotic disease (characterised by arteriosclerosis and structural heart disease),[8-10] which manifests clinically as heart failure and has a high incidence of sudden cardiac death.[2, 10]

Randomized trials of sodium-glucose co-transporter 2 (SGLT2) inhibitors have shown substantial reductions in adverse cardiovascular and renal outcomes in people with diabetic kidney disease.[11, 12, 13**, 14**] Recently the results of the Effect of Dapagliflozin on Renal Outcomes and Cardiovascular Mortality in Patients With Chronic Kidney Disease (DAPA-CKD) trial were announced.[15**, 16**] DAPA-CKD randomized 4304 (2906 [67.5%] with type 2 diabetes and 1398 [32.5%] without diabetes) participants with an estimated glomerular filtration rate (eGFR) ≥25 ≤75 mL/min/1.73m^2; urinary albumin:creatinine ratio (uACR) ≥200 ≤5000 mg/g; and on stable, maximum tolerated RAS inhibition to dapagliflozin orplacebo.[15**] Allocation to dapagliflozin (median 2.4 years follow up), reduced risk of the primary endpoint (defined as [i] >50% sustained decline in eGFR or ESKD or; [ii] renal or CVD mortality) by 39% (hazard ratio [HR] 0.61 [95% confidence interval (CI) 0.51-0.72]; P=0.000000028).[16**] The results were consistent in those with and without diabetes.[16**]

Dapagliflozin substantially reduced risk of hospitalisation for heart failure or CVD mortality by 39% (HR 0.71; 95% CI 0.55-0.92; P=0.0089) and all-cause mortality by 31% (HR 0.69; 95%

CI 0.53-0.88; P=0.0035).[16**] These data demonstrate SGLT2 inhibitors offer a new treatment option for improving renal outcomes in a range of patients with proteinuric CKD. However, the effects of SGLT2 inhibitors in individuals with lower GFR or smaller quantities of proteinuria remain uncertain and are currently being tested in the EMPA-KIDNEY trial which is ongoing.[17**]

Another therapeutic strategy has been to target the natriuretic peptide (NP) system. The NP system is a compensatory neurohormonal pathway that counter-regulates excess RAS and sympathetic nervous system activation in disease states with relative NP deficiency including CKD, hypertension and CVD. Enhancing the activity of NPs by inhibiting neprilysin (the enzyme responsible for breaking down NPs) could have therapeutic benefit.

Natriuretic peptides and neprilysin

Natriuretic peptides (NPs) are a family of peptides and three are present in humans: atrial (ANP), brain (BNP) and C-type (CNP).[18] ANP and BNP are synthesised in cardiac myocytes in response to atrial distension.[18] CNP is predominantly expressed in endothelial cells.[19]

NPs are formed as pre-pro-peptides.[18] Signal peptide cleavage forms pro-peptides which undergo further proteolytic cleavage, forming inactive N-terminal fragments NT-proANP$_{1-98}$ or NT-proBNP$_{1-76}$ and biologically active ANP$_{99-126}$ or BNP$_{1-32}$.[18, 20, 21] Renal ANP precursor expression produces urodilatin which regulates sodium and water excretion.[22] Cardiovascular effects of ANP include inhibition of endothelin production, proliferation of smooth muscle cells and myocardial hypertrophy.[18, 20]

Circulating concentrations of NT-proBNP rise in heart failure and are measured to assess disease activity. Actions of BNP include coronary vasodilatation, myocardial relaxation, proliferation of cardiac myocytes and inhibition of cardiac fibroblasts, all of which prevent cardiac remodelling, diastolic dysfunction and heart failure.[23] Both ANP and BNP also have anti-fibrotic and anti-inflammatory actions.[23]

NPs act (Figure 1) via natriuretic peptide receptors (NPRs). ANP and BNP act via NPR-A and CNP via NPR-B.[18, 19] NPRs are coupled to cyclic guanosine monophosphate-dependent signalling.[20, 24] NPs have a range of potentially beneficial actions and circulating levels are upregulated in disease states such as CKD and heart failure to counteract overactive harmful pathways (e.g., RAS and sympathetic nervous system).

NPs have several cardiorenal effects contributing to salt and water homeostasis and blood pressure control.[21, 25, 26] ANP and urodilatin regulate natriuresis and diuresis by inhibiting actions of angiotensin II (ATII), renin, aldosterone and anti-diuretic hormone. [20, 22, 27-29]

ANP increases renal perfusion through systemic vasodilatation, preferential pre-glomerular afferent arteriolar vasodilatation and post-glomerular efferent arteriolar vasoconstriction which increases intraglomerular capillary pressure, filtration fraction and GFR.[29, 30] ANP

counteracts ATII-induced mesangial cell contraction, thereby increasing capillary surface area for filtration and producing a diuresis.[31, 32] GFR is maintained despite increased distal sodium delivery by reduced sodium reabsorption and increased natriuresis.[33]

Animal models lacking the pro-ANP gene develop salt-sensitive hypertension (SSH) compared to the wild-type.[34, 35] Gene delivery of ANP to mice with SSH reduces blood pressure, renal injury, cardiac hypertrophy and stroke rates.[36, 37] Genetic variation in genes encoding NPs (*NPPA-NPPB*) or NP ratios (*POC1B-GALNT4*, *PPP3CC*) have been associated with reduced blood pressure and risk of hypertension.[38]

These findings suggest augmenting circulating NPs levels could improve clinical outcomes in hypertension, cardiovascular and CKD.

Neprilysin

Neprilysin or neutral endopeptidase (NEP) is a membrane-bound zinc-containing metalloproteinase[18, 39] with widespread tissue distribution. NEP is responsible for degrading NPs[39-42] and regulating a variety of other vasoactive peptides including ATII, bradykinin, endothelin-1, substance P, adrenomedullin and amyloid.[41-44]

NEP plays an important role in the formation and breakdown of the vasoconstrictor endothelin (ET) as it shares sequence homology with endothelin-converting enzyme (ECE).[41, 44-46] NEP inhibition (NEPi) attenuates the activity of ECE-1 which enhances actions of ANP.[41] The net effect of NEP depends on the balance between vasoconstrictor and vasodilatory peptides.

Infusions of recombinant NPs were associated with profound hypotension and lacked efficacy in randomized trials.[47, 48] The wide range of potentially therapeutic actions of NPs led to the development of NEP inhibitors.

Neprilysin inhibition

Candoxatrilat was one of the first NEP inhibitors to be tested in a range of patients with CKD, hypertension and heart failure. Short term studies demonstrated candoxatrilat reduced systemic blood pressure without changes in GFR or renal plasma flow.[49] However, chronic NEPi did not translate into clinically meaningful blood pressure reductions as NEPi impairs the degradation of ATII. Compensatory up-regulation of RAS and sympathetic nervous activity attenuated effects on NEPi on blood pressure and natriuresis.[44, 50, 51]

The beneficial renal and cardiovascular actions of NEPi were enhanced when combined with simultaneous RAS inhibition resulting in the development of combined NEP/RAS inhibitors.[20]

Combined NEP/RAS inhibitors

Combined NEP/RAS inhibitors were developed as potential treatments for renal and cardiovascular disease. NEPi was initially combined with ACEi forming vasopeptidase inhibitors (VPIs).[52] Omapatrilat was the most widely studied VPI.[43, 53, 54]

In animal models of hypertension, combined NEP/RAS inhibition resulted in greater reductions in blood pressure and vascular remodelling compared with isolated RAS inhibition.[45, 46, 55-58] In animal models of CKD and diabetic nephropathy, compared with ACEi, omapatrilat substantially reduced progression of proteinuria despite similar effects on blood pressure.[59-61] Micropuncture studies showed omapatrilat led to greater reductions in glomerular capillary pressure.[59] Renal histology revealed significantly greater reductions in glomerulosclerosis and tubulointerstitial fibrosis compared with ACEi-alone, suggesting combined NEP/ACE inhibition yielded greater renoprotection.[59-61]

In studies of healthy volunteers, omapatrilat produced marked ACEi (decreasing ATII) and reductions in systemic blood pressure.[54] Renal effects included decreased filtration fraction and renal vasodilatation without changes in GFR.[54] The data suggested combined

NEP/RAS inhibition could have favourable effects on renal function and preservation of GFR over isolated RAS inhibition.

The Omapatrilat Cardiovascular Treatment versus Enalapril (OCTAVE) trial, randomized 25,302 hypertensive patients and compared the effects of omapatrilat with enalapril on blood pressure and risk of angioedema.[62] At 8 weeks, omapatrilat significantly reduced systolic (3.6 mmHg; P<0.001) and diastolic (2.0 mmHg; P<0.001) blood pressure compared with enalapril. Despite these promising results, increasing reports emerged of unacceptable rates of angioedema with omapatrilat, compared with isolated RAS inhibition, in some cases requiring hospitalisation and mechanical ventilation.[62-64] Following the OCTAVE results, omapatrilat was withdrawn from development.[65]

ARBs have similar cardiorenal actions to ACEi but minimal effects on bradykinin and are therefore much less likely to cause angioedema.[66, 67] Since NEPi must be combined with simultaneous RAS blockade, ARNI's were developed, combining the beneficial effects of RAS inhibition with NEPi, without excess risk of angioedema (Figure 1).[66, 68, 69]

Figure 1: Mechanism of action of angiotensin receptor-neprilysin inhibitors

ACE = angiotensin converting enzyme; ANP = atrial natriuretic peptide; BNP = brain natriuretic peptide; CNP = C-type natriuretic peptide; NEP = neprilysin; NEPi = neprilysin inhibition; NPR = natriuretic peptide receptor; RAS = renin-angiotensin system

Angiotensin receptor-neprilysin inhibitor (ARNI)

Sacubitril/valsartan (previously LCZ696) was the first angiotensin receptor-neprilysin inhibitor (ARNI) to be developed. It combines an angiotensin receptor blocker (ARB) valsartan with an NEPi sacubitril.[69] On ingestion, sacubitril/valsartan is rapidly metabolised into its component drugs. Sacubitril undergoes further conversion to form the active NEPi, sacubitrilat.[68-70] 50-70% of sacubitril is excreted in the urine as sacubitrilat and the remainder in the faeces.[71]

Randomized trials including people with hypertension, heart failure and CKD have assessed the safety and efficacy of sacubitril/valsartan in these populations.

Effects of ARNI on blood pressure

In spontaneously hypertensive rats, sacubitril/valsartan demonstrated superior blood pressure lowering compared with valsartan and ameliorated cardiac hypertrophy, fibrosis, coronary vascular remodelling and endothelial dysfunction.[72]

In 1328 people with mild-to-moderate hypertension, at 8 weeks compared with valsartan, sacubitril/valsartan had superior diastolic (2.17 mmHg; 95% CI 1.06-3.28; P<0.0001) and systolic (4.20 mmHg; 95% CI -5.94 to -2.46; P<0.0001) blood pressure lowering.[73]

Whether such reductions in blood pressure translate into improved renal and CV outcomes in patients with CKD or hypertension remains unclear. Except in heart failure populations, no large-scale randomized clinical outcome trials have been performed with sacubitril/valsartan for hypertension or other conditions.

Renal effects of ARNI

In animal models of CKD, combined NEP/RAS inhibition significantly reduced proteinuria, renal ultrastructural and tubular injury markers of kidney damage compared with isolated RAS inhibition.[74, 75*] These effects occurred independently of blood pressure reductions and may be mediated by inhibiting inflammation and oxidative stress.[75*, 76*, 77*]

The UK Heart and Renal Protection (HARP)-III trial randomized 414 people with advanced CKD to sacubitril/valsartan or irbesartan, and assessed the short- to medium-term effects of sacubitril/valsartan on kidney function.[78] Eligibility required an eGFR ≥20 <45mL/min/1.73m^2, or eGFR ≥45 <60mL/min/1.73m^2 and uACR >20mg/mmol.[78]

At 12 months, there was no significant difference in measured GFR with sacubitril/valsartan vs irbesartan: between-group difference of 0.1 (SE 0.7) mL/min/1.73m²; P=0.86.[79*] Similarly there was no effect on eGFR (mean difference 0.1 mL/min/1.73m^2; 95% CI -0.5 to 0.7; P=0.66).[79*] Sacubitril/valsartan had no significant effect on uACR compared with irbesartan: study average difference -9% (95% CI -18 to 1; P=0.08).[79*] By contrast, trials in heart failure suggested allocation to sacubitril/valsartan was associated with modest increases in albuminuria compared to RASi alone.[80, 81]

Sacubitril/valsartan, compared with irbesartan, significantly reduced NT-proBNP and troponin I concentrations by 18% (95% CI 11-25) and 16% (95% CI 8-23) respectively.[79*] Mean systolic and diastolic blood pressure were reduced by 5.4 (95% CI -7.4 to -3.4; P <0.001) mmHg and 2.1 (95% CI -3.3 to -1.0; P <0.001) mmHg respectively.[79*] There were no major safety or tolerability concerns.[79*]

The Prospective comparison of ARNI with ACEi to Determine Impact on Global Mortality and morbidity in Heart Failure trial (PARADIGM-HF) trial, randomized 8442 patients with heart failure with reduced ejection fraction (HFrEF) to sacubitril/valsartan or enalapril.[81] Baseline eGFR was 67.7 mL/min/1.73m^2 and 36% had CKD (eGFR <60 ml/min/1.73m^2).[81] Amongst those with CKD, eGFR was 49±8 mL/min/1.73m^2 at screening.[82**] eGFR declined less in people allocated sacubitril/valsartan compared with enalapril (7.8 [95% CI 9.6-6.0] versus 10.2 [95% CI 12.1-8.3] mL/min/1.73m^2 respectively).[82**] Annual rate of eGFR decline was also slower (1.61 [95% CI -1.77 to -1.44] versus 2.04 [95% CI -2.21 to-1.88] mL/min/1.73m^2/year respectively; P<0.001).[82**]

Similar results were observed in the Prospective Comparison of ARNI with ARB Global Outcomes in HF with Preserved Ejection Fraction (PARAGON-HF) trial, randomized 4822 patients with heart failure with preserved ejection fraction (HFpEF) to sacubitril-valsartan or valsartan.[83*] Mean eGFR was 62.5±19 mL/min/1.73m^2 at baseline and 48.5% (2341/4822) had an eGFR of 30-60 mL/min/1.73m^2.[83*]

It is hypothesised that the absence of decline in GFR despite significant reductions in systemic blood pressure with sacubitril/valsartan in heart failure populations, may result from afferent arteriolar vasodilation with only a relative efferent arteriolar vasoconstriction (due to actions of NPs at this site). This increases intraglomerular capillary hydrostatic pressure and enables GFR to be maintained despite low systemic pressure.[84]

The data from heart failure trials suggest that ARNIs may have beneficial effects on kidney function. Despite the results from UK HARP-III showing a lack of effect on kidney function in patients with CKD, in the absence of a large-scale renal outcomes trial some uncertainty remains.

Nevertheless, the effects on blood pressure and cardiac biomarkers support the hypothesis that sacubitril/valsartan might reduce the risk of cardiovascular events (particularly those related to heart failure) among patients with CKD.

ARNI and cardiovascular outcomes

In PARADIGM-HF, sacubitril/valsartan, compared with enalapril, reduced risk of cardiovascular death or heart failure hospitalisation by 20% (914/4187 [21.8%] versus 1117/4212 [26.5%] respectively; HR 0.80 [95% CI 0.73-0.87]; P<0.001).[81] Cardiovascular mortality was reduced by 20% (558/4187 [13.3%] versus 693/4212 [16.5%]; HR 0.80 [95% CI 0.71-0.89]; P<0.001) and risk of hospitalisation for worsening heart failure by 21% (537/4187 [12.8%] versus 658/4212 [15.6%]; HR 0.79 [95% CI 0.71-0.89]; P<0.001).[81]

Analyses of the effect of sacubitril/valsartan across a range of systolic blood pressure values (<110 mmHg and >140 mmHg) demonstrated a consistent risk reduction in the primary outcome even in people with extremely low blood pressure (heart failure patients with extremely high risk of adverse outcomes), compared with enalapril.[85] The precise mechanism by which sacubitril/valsartan influences cardiovascular mortality is unknown

although suggested mechanisms include; counter-regulation of RAS and sympathetic nervous systems, upregulation of NPs and reduced myocardial fibrosis and remodelling.[86]

In PARAGON-HF, sacubitril/valsartan, compared with valsartan, had no significant effect on hospitalizations for heart failure and cardiovascular mortality (894 events in 526/2407 versus 1009 events in 557/2389 respectively; Rate Ratio 0.87 [95% CI 0.75-1.01]; P=0.06), or cardiovascular mortality (204/2407 [8.5%] versus 212/2389 [8.9%] respectively; HR 0.95 [95% CI 0.79-1.16]).[83*]

Several studies currently in progress are assessing the effects of sacubitril/valsartan in heart failure populations (Supplementary Table 1). The Efficacy and Safety of Sacubitril/Valsartan in Maintenance Haemodialysis Patients With Heart Failure (ESARHD-HF) is a 12 week open-label, randomized trial aiming to recruit 104 dialysis patients in China.[87] The primary outcome is change in left ventricular ejection fraction compared with benazepril.[87] No other trials of sacubitril/valsartan in people with CKD examining cardiovascular outcomes are currently in progress.

Conclusion

There is a clinical need for interventions that could both reduce risk of progression to ESKD and CVD in people with CKD, especially non-diabetic kidney disease. The UK HARP-III trial demonstrated ARNI with sacubitril/valsartan may offer a new therapeutic strategy to address the excess CVD risk in patients with CKD and, provides a strong rationale for undertaking a large-scale outcomes trials examining the effects of ARNI on cardiovascular outcomes in people with CKD. UK HARP-III did not exclude a potential benefit on kidney function and results from heart failure populations suggest sacubitril/valsartan may slow decline in kidney function and progression to ESKD, so renal outcomes would need to be considered in any future large-scale clinical outcomes trial in CKD.

Key points:

- Progressive chronic kidney disease (CKD), is associated with increased risk of cardiovascular disease characterised by structural heart disease and vascular stiffness manifesting as heart failure and sudden cardiac death.
- Combined neprilysin inhibition and angiotensin-II receptor blockade (angiotensin receptor-neprilysin inhibition [ARNI]) with sacubitril/valsartan reduces the risk of cardiovascular mortality in people with heart failure with reduced ejection fraction and possibly reduces progression of kidney disease.
- In people with advanced CKD, ARNI has no additional effect on kidney function or albuminuria compared with angiotensin receptor blockade (ARB)-alone.
- In people with CKD, ARNI substantially reduced blood pressure and cardiovascular biomarkers (NT-proBNP and troponin I) compared with ARB-alone.
- ARNI could provide substantial benefit on cardiovascular outcomes in people with advanced and progressive CKD.

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Conflicts of interest

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This trial showed SGLT2 inhibition with canagliflozin, compared with placebo, was associated with a reduced risk of progressive decline in kidney function and reduced albuminuria. These data suggested the drug could have renoprotection in people with type 2 diabetes mellitus.

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This trial was stopped early for efficacy and showed SGLT2 inhibition with canagliflozin compared with placebo reduced risk of progression of kidney disease to ESKD and cardiovascular mortality in people with type 2 diabetes mellitus. These data showed SGLT2 inhibitors provide cardiorenal protection in people with type 2 diabetes mellitus.

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This paper described the rationale for the DAPA-CKD trial. The trial was stopped early in March 2020 for efficacy, meeting both primary and all secondary outcomes in people with and without diabetic nephropathy.

16. **European Society of Cardiology. DAPA-CKD trial meets primary endpoint in patients with chronic kidney disease 2020 [Available from: https://www.escardio.org/The-ESC/Press-Office/Press-releases/DAPA.

The DAPA-CKD results were recently presented at the European Society of Cardiology meeting. SGLT2 inhibition with dapagliflozin compared with placebo, reduced risk of >50% sustained decline in eGFR or ESKD or renal or CVD mortality by 39%, with similar results in those with and without type 2 diabetes. The results also showed substantial reductions in all cause mortality.

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The paper details the mechanism of action of SGLT2 inhibition and outlines the trial rationale for the EMPA-KIDNEY trial. The trial examining the effects of SGLT2 inhibition with comparing empagliflozin vs placebo in people with non-diabetic nephropathy. The trial is currently ongoing and the results are eaglery awaited in view of the recent DAPA-CKD results.

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