LEARNING THE ROPES:

Targeting C-domain of Angiotensin Converting Enzyme and Nephilysin as a Potential New Therapeutic Strategy in Hypertension

RHÉURE ALVES-LOPES¹, EDWARD STURROCK²; RHIAN M TOUYZ³

¹ Institute of Cardiovascular and Medical Sciences, University of Glasgow, UK

² Institute of Infectious Disease and Molecular Medicine and Department of Integrative Biomedical Sciences, University of Cape Town, South Africa;

³ Research Institute of the McGill University Health Centre, McGill University, Canada.

DOI: 10.30824/2112-7

More than 30% of all deaths worldwide are due to cardiovascular disease, where overactivity of the renin-angiotensin-aldosterone system (RAAS) plays a key role. Activation of the RAAS leads to fibrosis, excessive vasoconstriction, reduced vasodilation, increased sodium and water retention, and cardiac hypertrophy. This has led to the development of drugs that inhibit key components of the RAAS to treat conditions including hypertension, where angiotensin-converting enzyme inhibitors (ACEi) are among the first-line treatments. Reduction of blood pressure induced by ACEi is mostly via reduction of angiotensin II (Ang II) levels, and reduction in bradykinin (BK) degradation. The anti-hypertensive effects of ACEi and angiotensin receptor blockers (ARB) are well established. However, some patients are non-responsive to these drugs, hence, there is a growing need for novel antihypertensive drugs^{1,2}.

Drugs targeting diverse components of the RAAS are used to treat cardiovascular diseases, where most of them are predominantly designed to antagonize Ang II effects. Increasing evidence indicates that in addition to Ang II, other components play an important role in the system, including neprilysin (NEP). NEP also controls BK degradation and inhibition of NEP causes an increase in BK levels. However, NEP inhibition alone is not able to sustain a reduction in blood pressure, probably because NEP also regulates other peptides involved in vascular control, including natriuretic peptides, Ang I, Ang II, ET-1, enkephalins, substance P, insulin, amyloid- β peptide, and gastrin^{1,2}. NEP inhibitors also have a short half-life.

There is similarity between the three-dimensional structures of ACE and NEP, which enabled the development of a single molecule able to target both

enzymes. Clinical trial data comparing antihypertensive effects of dual inhibition of ACE and NEP (omapatrilat) versus ACEi alone showed greater efficacy for omapatrilat. However, a large phase III clinical trial, demonstrated unwanted side effects, specifically angioedema with omapatrilat and accordingly the drug failed to obtain U.S. Food and Drug Administration (FDA) approval. Increased risk of angioedema induced by omapatrilat is associated with high levels of BK, probably because this drug inhibits NEP, aminopeptidase P, and ACE, all of which are involved in BK degradation. ACE has two distinct catalytic domains, N- and C-domains. The C-domain hydrolyzes angiotensin I (Ang I) and is primarily responsible for Ang II production. On the other hand, both domains contribute to the degradation of BK, and nonspecific inhibition of C- and N-domains, leads to excess BK formation, leading to adverse effects³.

To better explore the inhibition of NEP, it was thought that this class of drug could be combined with endothelin-converting enzyme-1 (ECE-1) inhibitors to treat cardiovascular conditions. This was based on the fact that the accumulation of ET-1, induced by NEP inhibition, would be prevented if ECE-1 was also inhibited. However, systemic blood pressure, heart rate, and cardiac output were unaffected by dual ECE-1/NEP inhibition, further highlighting the important role of the RAAS in blood pressure control⁴.

A third strategy was to combine a drug that simultaneously inhibits ACE, NEP and ECE-1. However, similar to what was observed with omapatrilat, pre-clinical data showed a significant accumulation of BK, and once again safety issues were a concern⁵. The complexity of this approach may cause many off-target

actions, and possibly serious undesired effects, and needs careful evaluation before testing this combination in humans.

Recognition of serious adverse effects associated with BK accumulation led to the development of sacubitril/valsartan (previously known as LCZ696). This combination reduces NEP activity via sacubitril while inhibiting the detrimental effects of Ang II via valsartan, with no effect on ACE, and therefore less impact on BK levels. Despite this drug having demonstrated clinical efficacy in the reduction of blood pressure in patients, this medication is approved for the treatment of heart failure only, and it is estimated that thousands of deaths could be prevented each year with the implementation of sacubitril/valsartan therapy to treat hypertension⁶. Despite its effective blood pressure-lowering effects, the drug is licensed primarily for heart failure and not for arterial hypertension.

Since the N- and C-domains of ACE have different functions, it was thought that targeting the domain only involved in Ang II production may be an interesting approach to regulate blood pressure without undesired

effects induced by N-domain inhibition including BK accumulation. With the development of a C-domain-selective ACEi, a derivative of lisinopril [lisW-S (lisinopril-tryptophan)], by substituting a tryptophan for the P2' proline⁷⁻⁹, a potential new strategy to treat hypertension was launched. The combination of lisW-S with a NEP inhibitor (sacubitril) in an animal model of hypertension reduced systolic blood pressure (SBP) similar to that of omapatrilat. However, unlike omapatrilat, which improved endothelial function, this was not observed for lisW-S + sacubitril. In addition, omapatrilat but not lisW-S + sacubitril caused plasma extravasation and vascular leakage, important processes that play a role in angioedema. Of significance, lisW-S + sacubitril, but not omapatrilat, increased plasma levels of BK inactive metabolites, suggesting that N-domain activity of ACE is preserved in animals treated with lisW-S. Together these findings indicate that lisW-S combined with sacubitril has the same beneficial effects as omapatrilat, but without the undesirable properties attributed to BK accumulation¹⁰. This combination provides potential new approaches in the treatment of hypertension and opens new avenues for development of novel anti-hypertensive drugs.

Figure 1. Pharmacological targets involving RAAS system and ECE in the treatment of hypertension. Evidence indicates that in addition to Ang II, other components play an important role in blood pressure control, including neprilysin (NEP) and ECE. All of them, somehow negatively modulates pro-hypertensive signalling pathway, however, undesired effects such as BK accumulation and angioedema, make it impossible for most of these drugs to be used therapeutically. With the development of a C-domain-selective ACEi (tryptophan), a potential new strategy to treat hypertension was launched: tryptophan combined with NEP inhibitor Sacubitril. ACE: Angiotensin Converting Enzyme; NEP: Neprilysin; ECE-1: Endothelin converting enzyme 1; AT1: angiotensin II type 1; LisW-S: Lisinopril-tryptophan.

REFERENCES

1. Oparil S, Acelajado MC, Bakris GL, Berlowitz DR, Cifkova R, Dominiczak AF, et al. Hypertension. *Nat Rev Dis Primers*. 2018;4:18014.
2. Arendse LB, Danser AHJ, Poglitsch M, Touyz RM, Burnett JC, Jr., Llorens-Cortes C, et al. Novel Therapeutic Approaches Targeting the Renin-Angiotensin System and Associated Peptides in Hypertension and Heart Failure. *Pharmacol Rev*. 2019;71(4):539-70.
3. Coats AJ. Omapatrilat--the story of Overture and Octave. *Int J Cardiol*. 2002;86(1):1-4.
4. Dickstein K, De Voogd HJ, Miric MP, Willenbrock R, Mitrovic V, Pacher R, et al. Effect of single doses of SLV306, an inhibitor of both neutral endopeptidase and endothelin-converting enzyme, on pulmonary pressures in congestive heart failure. *Am J Cardiol*. 2004;94(2):237-9.
5. Daull P, Benrezzak O, Arsenault D, Pheng LH, Blouin A, Cayer J, et al. Triple vasopeptidase inhibition normalizes blood pressure in conscious, unrestrained, and spontaneously hypertensive rats. *Am J Hypertens*. 2005;18(12 Pt 1):1606-13.
6. Fonarow GC, Hernandez AF, Solomon SD, Yancy CW. Potential Mortality Reduction With Optimal Implementation of Angiotensin Receptor Neprilysin Inhibitor Therapy in Heart Failure. *JAMA Cardiol*. 2016;1(6):714-7.
7. Denti P, Sharp SK, Kroger WL, Schwager SL, Mahajan A, Njoroge M, et al. Pharmacokinetic evaluation of lisinopril-tryptophan, a novel C-domain ACE inhibitor. *Eur J Pharm Sci*. 2014;56:113-9.
8. Schiering N, D'Arcy A, Villard F, Ramage P, Logel C, Cumin F, et al. Structure of neprilysin in complex with the active metabolite of sacubitril. *Sci Rep*. 2016;6:27909.
9. Burger D, Reudelhuber TL, Mahajan A, Chibale K, Sturrock ED, Touyz RM. Effects of a domain-selective ACE inhibitor in a mouse model of chronic angiotensin II-dependent hypertension. *Clin Sci (Lond)*. 2014;127(1):57-63.
10. Alves-Lopes R, Montezano AC, Neves KB, Harvey A, Rios FJ, Skiba DS, et al. Selective Inhibition of the C-Domain of ACE (Angiotensin-Converting Enzyme) Combined With Inhibition of NEP (Neprilysin): A Potential New Therapy for Hypertension. *Hypertension*. 2021;78(3):604-16.

Rhure Alves-Lopes - Rheure.lopes@glasgow.ac.uk

Edward Sturrock - edward.sturrock@uct.ac.za

Rhian Touyz - rhian.touyz@mcgill.ca