

Cardiac remodelling and RAS inhibition

Carlos M. Ferrario

Abstract: Risk factors such as hypertension and diabetes are known to augment the activity and tissue expression of angiotensin II (Ang II), the major effector peptide of the renin-angiotensin system (RAS). Overstimulation of the RAS has been implicated in a chain of events that contribute to the pathogenesis of cardiovascular (CV) disease, including the development of cardiac remodelling. This chain of events has been termed the CV continuum. The concept of CV disease existing as a continuum was first proposed in 1991 and it is believed that intervention at any point within the continuum can modify disease progression. Treatment with antihypertensive agents may result in regression of left ventricular hypertrophy, with different drug classes exhibiting different degrees of efficacy. The greatest decrease in left ventricular mass is observed following treatment with angiotensin converting enzyme inhibitors (ACE-Is), which inhibit Ang II formation. Although ACE-Is and angiotensin receptor blockers (ARBs) provide significant benefits in terms of CV events and stroke, mortality remains high. This is partly due to a failure to completely suppress the RAS, and, as our knowledge has increased, an escape phenomenon has been proposed whereby the human sequence of the 12 amino acid substrate angiotensin-(1-12) is converted to Ang II by the mast cell protease, chymase. Angiotensin-(1-12) is abundant in a wide range of organs and has been shown to increase blood pressure in animal models, an effect abolished by the presence of ACE-Is or ARBs. This review explores the CV continuum, in addition to examining the influence of the RAS. We also consider novel pathways within the RAS and how new therapeutic approaches that target this are required to further reduce Ang II formation, and so provide patients with additional benefits from a more complete blockade of the RAS.

Keywords: angiotensin-(1-12), angiotensin II, cardiac remodelling, cardiovascular continuum, renin-angiotensin system (RAS)

Introduction

Risk factors such as hypertension and diabetes are known to stimulate the production of angiotensin II (Ang II), the major effector peptide of the renin-angiotensin system (RAS) [Ferrario and Strawn, 2006]. The RAS plays a major role in the preservation of haemodynamic stability, with overstimulation of this system implicated in a chain of events that contribute to the pathogenesis of cardiovascular (CV) disease [Ferrario and Strawn, 2006]. Over the past two decades, a wealth of research has significantly improved our understanding of CV disease. In particular, we have gained invaluable insights into the chain of events that can ultimately lead to the development of target organ damage, end-organ failure and death [Dzau and Braunwald, 1991; Ferrario and Strawn, 2006]. This knowledge has resulted

in the hypothesis that CV disease occurs as part of a series of events that form a continuum, which occurs due to the presence of one or more risk factors [Dzau and Braunwald, 1991; Ferrario and Strawn, 2006]. These risk factors, which have been shown to have an additive effect, include hypertension, hyperglycaemia, smoking, hyperlipidaemia, left ventricular hypertrophy and obesity [Dzau and Braunwald, 1991; Yusuf *et al.* 2004; Ferrario and Strawn, 2006]. Activation of inflammatory mechanisms in response to the tissue injury amplified by the presence of one or more of these risk factors results in the initiation of enhanced RAS activity, which mediates an adaptive and maladaptive response [Ferrario and Strawn, 2006]. This in turn plays an important role in the pathophysiology of CV disease, with inflammation being a key mechanism in the

Ther Adv Cardiovasc Dis

2016, Vol. 10(3) 162–171

DOI: 10.1177/

1753944716642677

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Correspondence to:
Carlos M. Ferrario, MD,
FAHA, FASH, FACC
Wake Forest University
Health Science Center,
Winston Salem, NC 27157,
USA
cmf@cmflearningsystems.com

initiation, progression and clinical sequelae of CV disease [Ferrario and Strawn, 2006]. Intervention at any point along this chain of events has been proposed as a means of disrupting the underlying pathophysiology of CV disease and conferring cardioprotection [Ferrario and Strawn, 2006]. The pleiotropic actions of Ang II as a hormone, either mediating or modulating cellular signalling mechanisms stimulating trophic, profibrotic, pro-thrombotic and native immune responses [Harrison *et al.* 2011], places blockade of this system at the core of treatment approaches to diseases of the heart and blood vessels. This review examines the stages that exist within this CV continuum, with particular attention on cardiac remodelling and how intervention in the RAS may improve CV outcomes.

The CV continuum

The concept of CV disease existing as part of a continuum was first proposed in 1991 by Dzau and Braunwald in a consensus statement [Dzau and Braunwald, 1991]. This working group identified a range of risk factors for CV disease and proposed that intervention at any point within the continuum has the ability to modify the progression of CV disease [Dzau and Braunwald, 1991; Ferrario and Strawn, 2006].

The first stage in the CV continuum is the occurrence of risk factors that predispose to tissue injury, such as hypertension, increased low density lipoproteins (LDLs) and diabetes. Subsequent steps in the continuum see the progressive advance of heart and vascular disease as exemplified by the development of atherosclerosis, ischemic heart disease leading to left ventricular dysfunction. If unchecked, this leads to clinical events such as myocardial infarction (MI), stroke and cardiac remodelling. Failure to effectively treat the patient at any of these stages in the continuum results in ventricular hypertrophy and fibrosis followed by congestive heart failure or cerebrovascular disease. Ultimately, the patient develops endstage heart disease, brain damage and dementia, resulting in cerebro/CV death [Dzau and Braunwald, 1991].

The association between risk factors and the development of CV disease was investigated in the large-scale standardized, case-control INTERHEART study, which was conducted in 52 countries [Yusuf *et al.* 2004]. In the INTERHEART study, 15,152 patients admitted

with symptoms of acute MI were compared with 14,820 age-matched controls [Yusuf *et al.* 2004]. This study revealed that 90% of the population attributable risk (PAR) for acute MI resulted from the presence of at least one of 9 independent risk factors (tobacco smoking, elevated apolipoprotein A, hypertension, diabetes, abdominal obesity, psychosocial factors, low fruit and vegetable intake, low physical activity and alcohol consumption). The effect of these risk factors was shown to be additive, with a greater risk of CV events occurring as the number of risk factors increased. For example, the combination of current smoking, hypertension and diabetes was shown to account for 53% of the PAR [Yusuf *et al.* 2004].

The mechanisms of action underlying the progression of the CV continuum have been attributed to the actions of Ang II, with the oxidative stress caused by the presence of risk factors producing an inflammatory response that favours a high expression of Ang II [Unger, 2002; Dell'Italia, 2011]. Acting through the Ang II type 1 (AT1) receptor, Ang II stimulates vascular remodelling, leading to increased blood pressure (BP) and contributing to chronic disease pathology by promoting vascular growth and proliferation, endothelial dysfunction and cellular damage due to increased accumulation of radical oxygen species [Dell'Italia, 2011]. Ang II is involved at every step in the process of the progression from atherosclerosis, coronary atherothrombotic disease, MI, remodelling and heart failure to end-stage heart disease [Dell'Italia, 2011]. Ang II has also been shown to exert a wide variety of deleterious effects that are mediated either directly, or through a number of signal-transduction pathways that result in cellular proliferation, increased oxidative stress and reduced nitric oxide levels [Morawietz *et al.* 2006]. Earlier suggestions of a link between the RAS and activation of immune mechanisms [Chatelain and Ferrario, 1978; Chatelain *et al.* 1980] leading to cardiac and vascular damage have been demonstrated through the provocative findings that Ang II is a modulator of immune mechanisms in hypertension [Harrison *et al.* 2011].

Cardiac remodelling

A meeting held in 1998, the International Forum on Cardiac Remodelling, aimed to discuss the basic mechanisms of cardiac remodelling and to provide a consensus on key concepts and definitions underlying this process [Cohn *et al.* 2000].

According to Cohn and colleagues, cardiac remodelling can be defined as 'genome expression, molecular, cellular and interstitial changes that are manifested clinically as changes in size, shape and function of the heart after cardiac injury' [Cohn *et al.* 2000]. Cardiac remodelling has been linked to a worsening of cardiac function and is one of the mechanisms that perpetuate CV disease progression, making it a potential therapeutic target for intervention [Cohn *et al.* 2000].

The left ventricular hypertrophy associated with cardiac remodelling normally begins within the first few hours after a MI [Cohn *et al.* 2000]. A number of factors can influence the speed of cardiac remodelling, including hemodynamic load and neurohormonal activation [Cohn *et al.* 2000]. In addition, ischaemia, cellular necrosis and apoptosis may play a role [Cohn *et al.* 2000]. It is a physiological and pathological condition that may occur following MI, pressure overload (e.g. aortic stenosis, hypertension), inflammatory heart muscle disease (i.e. myocarditis), idiopathic dilated cardiomyopathy or volume overload due to valvular regurgitation [Cohn *et al.* 2000]. The myocyte is the principal cardiac cell involved in the remodelling process, altering the shape of the heart, making it more spherical and less elliptical, increasing left ventricular volume and ventricular mass [Cohn *et al.* 2000]. Other components believed to be involved in cardiac remodelling include the interstitium, fibroblasts, collagen and coronary vasculature [Cohn *et al.* 2000].

Although the exact mechanisms underlying all the pathways and cells involved in cardiac remodelling have not been fully elucidated, the following hypothesis has been proposed by the members of the International Forum on Cardiac Remodelling: stretch within myocytes results in local noradrenaline activity together with angiotensin and endothelin release [Cohn *et al.* 2000]. This in turn stimulates myocyte hypertrophy due to the expression of altered proteins and results in a further deterioration in cardiac function. Increased neurohormonal activation, together with increased aldosterone and cytokine activation, stimulates collagen synthesis leading to fibrosis and remodelling of the extracellular matrix [Cohn *et al.* 2000]. Clinical studies have demonstrated that therapeutic agents such as angiotensin converting enzyme inhibitors (ACE-Is), Ang II receptor blockers (ARBs) and beta-blockers can modify the process of cardiac remodelling in addition to their other clinically

relevant benefits in reducing morbidity and mortality in patients with CV disease [Verdecchia *et al.* 1998; Cohn *et al.* 2000; Dell'Italia, 2011]. Taken together, these findings implicate the RAS in the mechanisms underlying cardiac remodelling.

Effects of Ang II

The key stage in the effector mechanisms of the RAS is the binding of Ang II to AT1 receptors. This initiates a signal transduction cascade that results in enhanced collagen synthesis by cardiac fibroblasts, causing fibrosis and myocyte hypertrophy, both of which underlie the development of cardiac hypertrophy (Figure 1) [Krenning *et al.* 2010]. Ang II has been found to display paracrine, intracrine and endocrine influences on cellular signalling pathways (Figure 2). Intracrine actions include the ability of intracellular renin, if present, to alter the electrical properties of the cardiac cell membrane by shortening of the action potential and reducing the refractoriness period [De Mello, 2013]. These changes occur due to an increase in the total potassium current within the cell, which results in concomitant changes in heart excitability. It has been proposed that these intracrine effects of Ang II might be of particular importance during pathological conditions such as MI, when heart excitability is already enhanced [De Mello, 2013]. Ang II has also been shown to increase the percentage and velocity of myocyte shortening, increase the velocity of myocyte re-lengthening and increase peak systolic transient calcium levels [Zhou *et al.* 2015].

A report presenting hormonal data obtained during the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS) [The Consensus Trial Study Group, 1987] evaluated the relationship between hormonal activation in heart failure and prognosis; an association between upregulation of ACE and the evolution of heart failure was noted [Swedberg *et al.* 1990]. Similar findings were reported in a study conducted by Hirsch and colleagues, who noted that the renal effects of ACE may play a role in heart failure [Hirsch *et al.* 1992]. In contrast, the limited ability of ACE-Is in heart failure progression and cardiac Ang II suppression may be related to the more prominent role of chymase in human Ang II production from angiotensin I (Ang I) [Zablocki and Sadoshima, 2010; Ferrario *et al.* 2014]. Thus, it is possible that an 'escape phenomenon' occurs with chronic Ang II suppression which allows

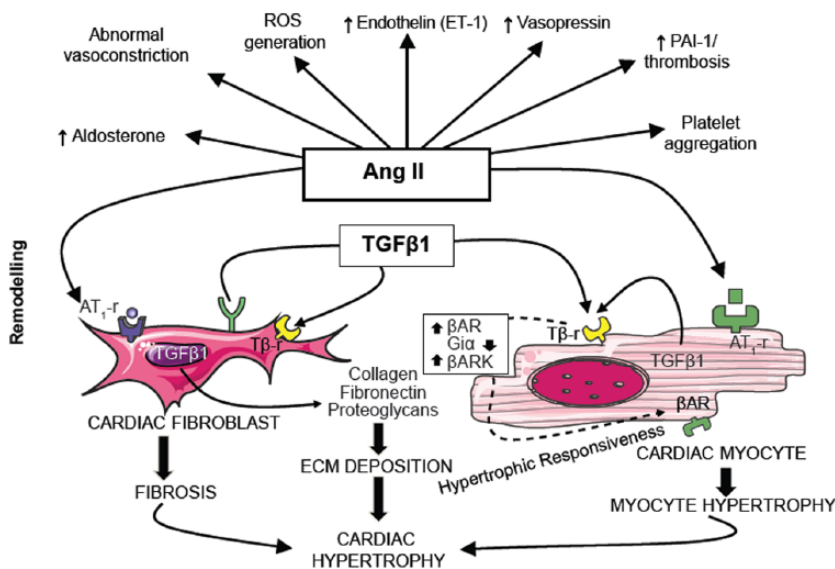


Figure 1. Angiotensin II drives the mechanisms underlying cardiac remodelling. Ang II, angiotensin II; AT₁-r, angiotensin II type 1 receptor; βAR, β-adrenergic receptor; βARK, β-adrenergic receptor kinase; ECM, extracellular matrix; PAI-1, plasminogen activator inhibitor-1; ROS, reactive oxygen species; Tβ-r, transforming growth factor β1.

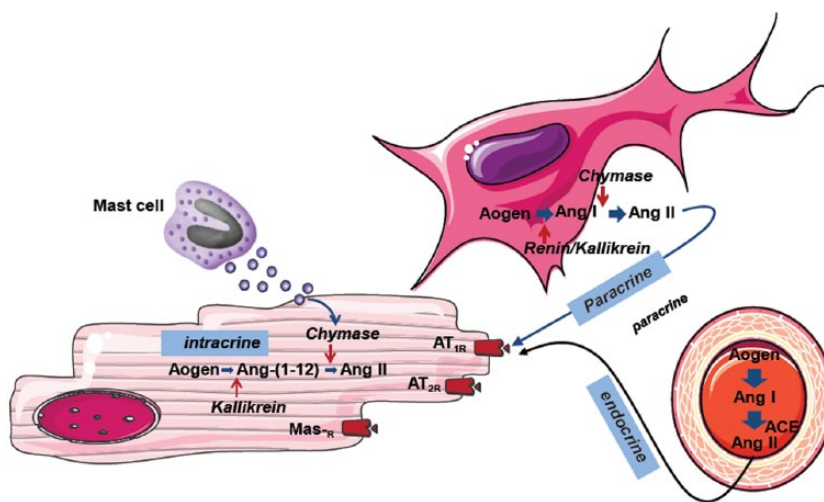


Figure 2. Intracrine, paracrine and endocrine signalling mechanisms of angiotensin II. ACE, angiotensin converting enzyme; Ang, angiotensin; AT_{1R}, angiotensin II type 1 receptor; AT_{2R}, angiotensin II type 2 receptor; Mas-R, Mas receptor.

Ang II to return to normal levels [Zablocki and Sadoshima, 2010].

Inhibition of the RAS

In a transgenic rat model of renin-dependent hypertension, direct renin inhibition by means of AT1 receptor blockade has been shown to reduce both cardiac hypertrophy and cardiac remodelling [Whaley-Connell *et al.* 2013]. Interestingly, a

reduction in interstitial cardiac fibrosis was also observed and it is thought that this may contribute to the observed reduction in hypertrophy [Whaley-Connell *et al.* 2013]. Different anti-RAS approaches act at different points within the system (Figure 3). For example, the direct renin inhibitor, aliskiren, acts early in the RAS pathway to block the hydrolysis of angiotensinogen to Ang I by the enzyme renin [Pilz *et al.* 2005; Ferrario, 2010; Esteras *et al.* 2015]. Further down the RAS

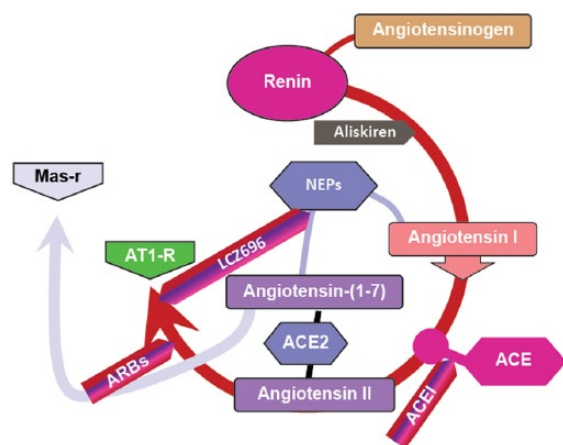


Figure 3. Target sites for the therapeutic inhibition of the renin–angiotensin system.

ACE, angiotensin converting enzyme; AT1-R, angiotensin II type 1 receptor; ARBs, angiotensin receptor blockers; Mas-r, Mas receptor.

pathway, ACE-Is prevent the hydrolysis of Ang I to the key effector of the pathway, Ang II [Esteras *et al.* 2015]. The effector actions of Ang II are inhibited by ARBs, which block the binding of Ang II to AT1 receptors [Esteras *et al.* 2015]. In addition, a novel combination of an AT1 receptor antagonist with an inhibitor of cardiac atrial natriuretic peptide metabolism within a single entity has shown superior efficacy over ACE-Is, and so may become a major pillar of future heart failure treatment approaches [McMurray *et al.* 2014]. Valsartan/sacubitril (previously known as LCZ696) is an example of an angiotensin receptor neprilysin inhibitor containing an ARB as well as a neprilysin inhibitor that acts to increase circulating levels of natriuretic peptides [Segura *et al.* 2013]. Another dual inhibitor, omapatrilat, combined ACE and neprilysin inhibition, but use of this combination has been associated with increased levels of angioedema [Buggey *et al.* 2015].

It has been suggested that treatment with antihypertensive agents may result in regression of left ventricular hypertrophy, with different drug classes exhibiting different degrees of hypertrophy regression [Schmieder *et al.* 1996]. A meta-analysis of 39 randomized controlled trials examined the ability of various antihypertensive agents to reduce left ventricular hypertrophy. This analysis demonstrated that greater reductions in BP were associated with a more marked decrease in left ventricular hypertrophy, as was a longer duration of treatment [Schmieder *et al.* 1996]. The greatest decrease in left ventricular mass and wall thickness was

observed with ACE-Is (13%) compared with reductions of 9% with calcium channel blockers, 7% with diuretics and 6% with beta-blockers [Schmieder *et al.* 1996]. However, despite the efficacy of ACE inhibition in terms of BP lowering and interruption of the CV continuum, there is a growing body of evidence that these agents may not produce a sustained inhibition of Ang II synthesis [Garg and Yusuf, 1995; Ferrario, 2010; Ferrario *et al.* 2014]. Rather, an escape phenomenon which permits synthesis of Ang II *via* an alternative pathway may ultimately allow the RAS to overcome the effects of ACE-Is [Garg and Yusuf, 1995; Ferrario, 2010; Ferrario *et al.* 2014]. In addition, Ang II intracrine actions will not be affected by either ACE-Is or ARBs as these drugs act preferentially at the level of the cell membrane.

Clinical studies investigating the inhibition of the RAS

Although clinical trial data indicate that ACE-I and ARB therapies provide a significant benefit when compared with placebo in terms of endpoints such as MI, stroke, congestive heart failure, hospitalization for CV events and CV-related mortality, the incidence of these events remains high [The Consensus Trial Study Group, 1987; SOLVD Investigators, 1991; Yusuf *et al.* 2000; Dahlof *et al.* 2002; Granger *et al.* 2003]. The Heart Outcomes Prevention Evaluation (HOPE) Trial assessed the role of the ACE-I, ramipril, in patients who did not have left ventricular dysfunction or heart failure, but nonetheless were considered to be at high risk for CV events [Yusuf *et al.* 2000]. Treatment with ramipril reduced the rate of death from CV causes when compared with placebo (6.1% *versus* 8.1%, respectively). A reduction in the relative risk of MI, stroke, cardiac arrest and heart failure was also observed in those patients who received ramipril [Yusuf *et al.* 2000]. However, 14% of the patients in the treatment group still experienced a CV-related event compared with 17.8% of placebo-treated patients [Yusuf *et al.* 2000]. In other words, the residual risk for events was substantially higher than the benefit.

The Studies of Left Ventricular Dysfunction (SOLVD) trial compared the ACE-I enalapril with placebo in patients with congestive heart failure who were receiving conventional treatment [SOLVD Investigators, 1991]. The addition of enalapril to conventional treatment significantly

reduced mortality and hospitalization for heart failure in these patients. SOLVD demonstrated that 35.2% (452) of patients who were receiving enalapril died compared with 39.7% (510) of placebo-treated patients. Therefore, although the addition of this treatment added benefit, a considerable number of enalapril-treated patients did not survive [SOLVD Investigators, 1991]. Similarly, in the CHARM-Alternative (Candesartan in Heart failure – Assessment of Reduction in Mortality and Morbidity) trial, heart failure patients with ACE-I intolerance were randomised to receive either placebo or the ARB candesartan (target dose, 32 mg per day) [Pfeffer *et al.* 2003b]. During a median follow up of 33.7 months, hospitalization or CV-related death was reported in 33% ($n = 334$) of candesartan patients *versus* 40% ($n = 406$) of placebo patients. As in the SOLVD study, although active treatment offered clinical benefit when compared with placebo, a marked number of patients did not survive, or experienced an unfavourable outcome [Granger *et al.* 2003]. The Losartan Intervention For Endpoint reduction in hypertension study (LIFE) compared the ARB losartan with the beta-blocker atenolol in 9,193 individuals with essential hypertension and left ventricular hypertrophy [Dahlof *et al.* 2002]. This study noted fewer deaths and a lower rate of CV morbidity in those patients who received losartan compared with atenolol. However, there were still 204 deaths amongst patients in the losartan arm of the LIFE study [Dahlof *et al.* 2002]. It should be noted, however, that two important clinical trials have demonstrated significant benefits of the ARB valsartan in reducing the risk of hospital admissions for worsening heart failure, and death [Cohn and Tognoni, 2001] and evolution post-MI [Pfeffer *et al.* 2003a].

As previously discussed, cardiac tissue hypertrophy is indicative of cardiac damage. Pilz and colleagues [Pilz *et al.* 2005] examined the degree of cardiac hypertrophy occurring during treatment with antihypertensive agents in an animal model of hypertension using echocardiography to measure left ventricular wall thickness and cardiac hypertrophy index (Figure 4). The aim of this study was to test the hypothesis that the direct renin inhibitor, aliskiren, and the ARB, valsartan, possess the ability to ameliorate the cardiac and renal damage observed in this animal model. Compared with untreated animals, both aliskiren and valsartan reduced systolic BP and albuminuria, and prevented cardiac hypertrophy [Pilz *et al.* 2005]. Clinical data have also demonstrated that aliskiren, at a dose of 300 mg, was as effective as

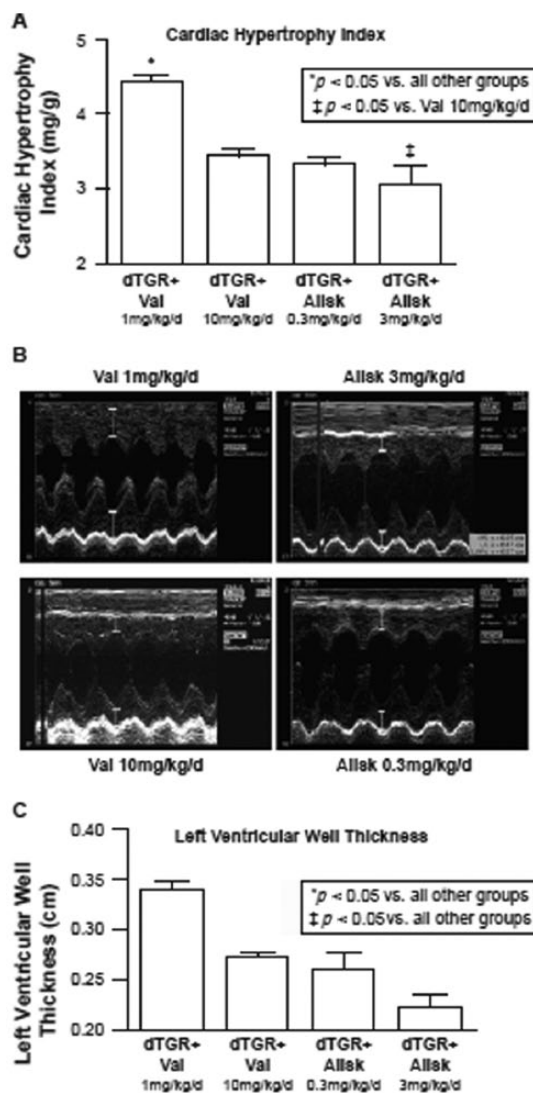


Figure 4. Comparative effects of valsartan (Val) and aliskiren (Allsk) in double transgenic rats (dTGR+) in terms of cardiac hypertrophic index (A, top panel), M-mode echocardiography (B, middle panel) and left ventricular dimensions (C, bottom panel). Aliskiren has been shown to prevent left ventricular hypertrophy and reduce left ventricular mass in an animal model of hypertension [Pilz *et al.* 2005]. Allsk, aliskiren; TGR, double transgenic rats; Val, valsartan.

the ARB losartan (100 mg) in promoting left ventricular mass regression in 465 patients with hypertension and increased ventricular wall thickness [Solomon *et al.* 2009]. Reduction in left ventricular mass in those who received aliskiren in combination with losartan was not significantly different from those who received losartan monotherapy [Solomon *et al.* 2009]. Thus, direct renin inhibition appears to be as effective as ARB treatment in attenuating the cardiac hypertrophy observed in hypertensive patients.

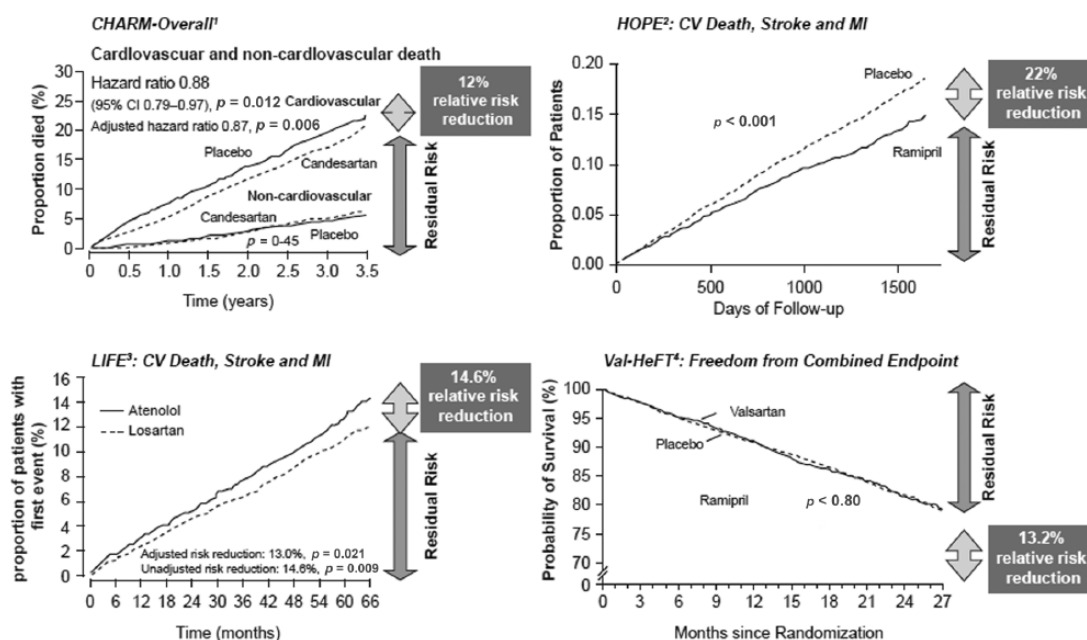


Figure 5. Plots of the Kaplan–Meier for cardiovascular end-points of four major trials comparing active treatment to corresponding placebos. Despite the benefits of ACE-Is and ARBs in terms of reducing hypertension, a residual risk of morbidity and mortality remains. Superscripts on the named-trials denote corresponding citations [¹Pfeffer *et al.* 2003b; ²Yusuf *et al.* 2000; ³Dahlöf *et al.* 2002; ⁴Cohn and Tognoni, 2001]. ACE-Is, angiotensin converting enzyme inhibitors; ARBs, angiotensin receptor blockers; CI, confidence interval; CV, cardiovascular; MI, myocardial infarction.

It is therefore apparent, that despite adequate treatment of hypertension, a residual risk of morbidity and mortality remains in those patients who are treated with either ACE-Is or ARBs. Several clinical studies, including CHARM, HOPE, LIFE and Val-HeFT have demonstrated considerable mortality rates in patients receiving anti-RAS medication (Figure 5) [Yusuf *et al.* 2000; Cohn and Tognoni, 2001; Dahlöf *et al.* 2002; Pfeffer *et al.* 2003b]. Moreover, studies have failed to produce clear evidence for the benefit of any particular drug class or classes that would support their recommendation for the treatment of either older or younger adults [Blood Pressure Lowering Treatment; Turnbull *et al.* 2008]. Thus, despite evidence for the involvement of Ang II in the pathogenesis of CV disease, the long-term use of RAS inhibitors in the treatment of patients with CV disease has not proved as effective as originally hoped, due to a partial failure to suppress the actions of Ang II within the cell itself [Ferrario *et al.* 2005].

Improved understanding of the RAS has presented novel targets

The observed loss of efficacy of ACE-Is over time has resulted in the hypothesis that an escape

phenomenon provides an alternative pathway for the synthesis of Ang II [Garg and Yusuf, 1995; Ferrario, 2010; Ferrario *et al.* 2014]. Angiotensin-(1-12) (Ang-(1-12)) is a novel 12 amino acid peptide substrate that was initially isolated from the rat intestine [Nagata *et al.* 2006]. In the presence of the Mast cell protease, chymase, Ang-1(1-12) is converted directly into Ang II in humans [Ahmad *et al.* 2011].

Investigations have shown that Ang-(1-12) is abundant in a wide range of organs and tissues including the small intestine, spleen, kidneys, heart and liver. Furthermore, it demonstrated the ability to constrict aortic strips *in vitro* and increase BP in rats, effects which were abolished in the presence of either the ACE-I captopril or the ARB candesartan [Nagata *et al.* 2006]. Following on from these findings, studies conducted by our research group at Wake Forest University School of Medicine have highlighted differences between humans and rodents regarding the enzymatic pathway responsible for the metabolism of Ang-(1-12) [Ferrario *et al.* 2014]. In addition, Ang-(1-12) has been shown to function as a non-renin alternative precursor for the local tissue production of Ang II, which facilitates

intracrine or paracrine RAS functions [Jessup *et al.* 2008; Ferrario *et al.* 2014].

Targeting of Ang-(1-12)

Characterization of the expression and localization of Ang-(1-12) has shown that it is predominantly found in ventricular myocytes and the proximal, distal and collecting renal tubules within the deep cortical and outer medullary zones [Jessup *et al.* 2008]. Moreover, comparisons of the expression of Ang-(1-12) in hypertensive rats and normotensive rats revealed significantly higher levels of Ang-(1-12) in the heart and significantly lower levels in the kidneys of hypertensive rats [Jessup *et al.* 2008]. The metabolism of Ang-(1-12) has been investigated in plasma membranes isolated from human atrial appendage tissue [Ahmad *et al.* 2011]. In the absence of any RAS inhibitor, Ang-(1-12) was converted into Ang I, Ang II, angiotensin-(1-7) and angiotensin-(1-4), with the primary metabolism occurring *via* chymase to produce Ang II. In parallel to these findings, Ahmad and colleagues also used immunohistochemistry to demonstrate significant chymase levels primarily localized within the atrial cardiac myocytes [Ahmad *et al.* 2011].

Cardiac ischaemia and reperfusion injury occurs as the consequence of an acute increase in oxidative and inflammatory stress during reperfusion. The outcome of this injury is the death of cardiac myocytes. Mast cells normally exist in an intact form within the myocardium, but become activated releasing enzymes, such as chymase, in response to acute stress. Following ischaemia/reperfusion cardiac injury, degranulation of mast cell contents into the interstitium is common [Zheng *et al.* 2014]. Using a preclinical model, Zheng and colleagues investigated the role of the Mast cell protease, chymase, in cardiomyocyte injury following ischaemia/reperfusion cardiac injury. Pretreatment with a specific oral chymase inhibitor significantly attenuated loss of laminin, focal adhesion complex disruption and release of troponin I into the circulation. Further immunohistochemical analyses demonstrated a high level of chymase within cardiomyocytes after ischaemia/reperfusion injury [Zheng *et al.* 2014]. Taken together, these findings provide evidence for a secondary pathway within the RAS that can enable the system to overcome blockade with traditional ACE-Is to continue to produce Ang II, thus permitting the effects of the RAS on the CV continuum to continue unchecked.

Conclusion

Research over the last few years has revealed that CV disease exists as a continuum, with treatment at any point within this process capable of modifying disease progression. Several studies have shown that left ventricular hypertrophy is an independent risk factor for mortality from CV disease. Moreover, this can be corrected by the use of treatments to inhibit the RAS, which will in turn, modulate the development of left ventricular hypertrophy and so interrupt the CV continuum. However, escape mechanisms mean that commonly used RAS inhibitors do not fully prevent the formation and actions of intracellular Ang II, which has itself been implicated in the process of cardiac remodelling. These escape mechanisms represent a novel intracrine pathway, whereby chymase is able to act within myocytes to convert angiotensin-(1-12) into Ang II. Therefore, new therapeutic approaches that target this novel pathway are required in order to reduce Ang II formation and so provide patients with further benefits from a more complete blockade of the RAS.

Acknowledgements

Editorial assistance was provided by Sarah Birch of Novartis Ireland Ltd., Dublin, Ireland.

Funding

The author acknowledges that his studies regarding novel angiotensins is supported by a grant (2P01 HL-051952) from the National Heart, Lung and Blood Pressure of the National Institutes of Health.

Conflict of interest statement

The author declares no conflicts of interest in preparing this article.

References

- Ahmad, S., Simmons, T., Varagic, J., Moniwa, N., Chappell, M. and Ferrario, C. (2011) Chymase-dependent generation of angiotensin II from angiotensin-(1-12) in human atrial tissue. *PLoS One* 6: e28501.
- Buggey, J., Mentz, R., Devore, A. and Velazquez, E. (2015) Angiotensin receptor neprilysin inhibition in heart failure: mechanistic action and clinical impact. *J Card Fail* 21: 741–750.
- Chatelain, R. and Ferrario, C. (1978) Biphasic changes in thymus structure during evolving renal hypertension. *Clin Sci Mol Med* 55: 149–156.
- Chatelain, R., Vessey, A. and Ferrario, C. (1980) Lymphoid alterations and impaired T lymphocyte

- reactivity in experimental renal hypertension. *J Lab Clin Med* 95: 737–747.
- Cohn, J., Ferrari, R. and Sharpe, N. (2000) Cardiac remodeling – concepts and clinical implications: a consensus paper from an International Forum on Cardiac Remodeling. *J Am Coll Cardiol* 35: 569–582.
- Cohn, J. and Tognoni, G. (2001) A randomized trial of the angiotensin-receptor blocker valsartan in chronic heart failure. *N Engl J Med* 345: 1667–1675.
- Dahlof, B., Devereux, R., Kjeldsen, S., Julius, S., Beevers, G., De Faire, U. *et al.* (2002) Cardiovascular Morbidity and mortality in the Losartan Intervention for Endpoint Reduction in Hypertension Study (LIFE): a randomised trial against atenolol. *Lancet* 359: 995–1003.
- De Mello, W. (2013) Intracellular renin alters the electrical properties of the intact heart ventricle of adult Sprague–Dawley rats. *Regul Pept* 181: 45–49.
- Dell'Italia, L. (2011) Translational success stories: angiotensin receptor 1 antagonists in heart failure. *Circ Res* 109: 437–452.
- Dzau, V. and Braunwald, E. (1991) Resolved and unresolved issues in the prevention and treatment of coronary artery disease: a workshop consensus statement. *Am Heart J* 121: 1244–1263.
- Esteras, R., Perez-Gomez, M., Rodriguez-Osorio, L., Ortiz, A. and Fernandez-Fernandez, B. (2015) Combination use of medicines from two classes of renin–angiotensin system blocking agents: risk of hyperkalemia, hypotension, and impaired renal function. *Ther Adv Drug Saf* 6: 166–176.
- Ferrario, C. (2010) Addressing the theoretical and clinical advantages of combination therapy with inhibitors of the renin–angiotensin–aldosterone system: antihypertensive effects and benefits beyond BP control. *Life Sci* 86: 289–299.
- Ferrario, C., Ahmad, S., Nagata, S., Simington, S., Varagic, J., Kon, N. *et al.* (2014) An evolving story of angiotensin-II-forming pathways in rodents and humans. *Clin Sci* 126: 461–469.
- Ferrario, C., Jessup, J., Chappell, M., Averill, D., Brosnihan, K., Tallant, E. *et al.* (2005) Effect of angiotensin-converting enzyme inhibition and angiotensin II receptor blockers on cardiac angiotensin-converting enzyme 2. *Circulation* 111: 2605–2610.
- Ferrario, C. and Strawn, W. (2006) Role of the Renin–angiotensin–aldosterone system and proinflammatory mediators in cardiovascular disease. *Am J Cardiol* 98: 121–128.
- Garg, R. and Yusuf, S. (1995) Overview of randomized trials of angiotensin-converting enzyme inhibitors on mortality and morbidity in patients with heart failure. *JAMA* 273: 1450–1456.
- Granger, C., McMurray, J., Yusuf, S., Held, P., Michelson, E., Olofsson, B. *et al.* (2003) Effects of candesartan in patients with chronic heart failure and reduced left-ventricular systolic function intolerant to angiotensin-converting-enzyme inhibitors: the CHARM-Alternative trial. *Lancet* 362: 772–776.
- Harrison, D., Guzik, T., Lob, H., Madhur, M., Marvar, P., Thabet, S. *et al.* (2011) Inflammation, immunity, and hypertension. *Hypertension* 57: 132–140.
- Hirsch, A., Talsness, C., Smith, A., Schunkert, H., Ingelfinger, J. and Dzau, V. (1992) Differential effects of captopril and enalapril on tissue renin–angiotensin systems in experimental heart failure. *Circulation* 86: 1566–1574.
- Jessup, J., Trask, A., Chappell, M., Nagata, S., Kato, J., Kitamura, K. *et al.* (2008) Localization of the novel angiotensin peptide, angiotensin-(1-12), in heart and kidney of hypertensive and normotensive rats. *Am J Physiol Heart Circ Physiol* 294: H2614–H2618.
- Krenning, G., Zeisberg, E. and Kalluri, R. (2010) The origin of fibroblasts and mechanism of cardiac fibrosis. *J Cell Physiol* 225: 631–637.
- McMurray, J., Packer, M., Desai, A., Gong, J., Lefkowitz, M., Rizkala, A. *et al.* (2014) Angiotensin-neprilysin inhibition *versus* enalapril in heart failure. *N Engl J Med* 371: 993–1004.
- Morawietz, H., Rohrbach, S., Rueckschloss, U., Schellenberger, E., Hakim, K., Zerkowski, H. *et al.* (2006) Increased cardiac endothelial nitric oxide synthase expression in patients taking angiotensin-converting enzyme inhibitor therapy. *Eur J Clin Invest* 36: 705–712.
- Nagata, S., Kato, J., Sasaki, K., Minamino, N., Eto, T. and Kitamura, K. (2006) Isolation and identification of proangiotensin-12, a possible component of the renin–angiotensin system. *Biochem Biophys Res Commun* 350: 1026–1031.
- Pfeffer, M., McMurray, J., Velazquez, E., Rouleau, J., Kober, L., Maggioni, A. *et al.* (2003a) Valsartan, captopril, or both in myocardial infarction complicated by heart failure, left ventricular dysfunction, or both. *N Engl J Med* 349: 1893–1906.
- Pfeffer, M., Swedberg, K., Granger, C., Held, P., McMurray, J., Michelson, E. *et al.* (2003b) Effects of candesartan on mortality and morbidity in patients with chronic heart failure: the CHARM-Overall programme. *Lancet* 362: 759–766.
- Pilz, B., Shagdarsuren, E., Wellner, M., Fiebeler, A., Dechend, R., Grätze, P. *et al.* (2005) Aliskiren, a human renin inhibitor, ameliorates cardiac and renal

- damage in double-transgenic rats. *Hypertension* 46: 569–576.
- Schmieder, R., Martus, P. and Klingbeil, A. (1996) Reversal of left ventricular hypertrophy in essential hypertension. A meta-analysis of randomized double-blind studies. *JAMA* 275: 1507–1513.
- Segura, J., Salazar, J. and Ruilope, L. (2013) Dual neurohormonal intervention in CV disease: angiotensin receptor and neprilysin inhibition. *Expert Opin Investig Drugs* 22: 915–925.
- Solomon, S., Appelbaum, E., Manning, W., Verma, A., Berglund, T., Lukashevich, V. *et al.* (2009) Effect of the direct renin inhibitor aliskiren, the angiotensin receptor blocker losartan, or both on left ventricular mass in patients with hypertension and left ventricular hypertrophy. *Circulation* 119: 530–537.
- SOLVD Investigators (1991) Effect of enalapril on survival in patients with reduced left ventricular ejection fractions and congestive heart failure. *N Engl J Med* 325: 293–302.
- Swedberg, K., Eneroth, P., Kjeksus, J. and Wilhelmssen, L. (1990) Hormones regulating cardiovascular function in patients with severe congestive heart failure and their relation to mortality. *Circulation* 82: 1730–1736.
- The Consensus Trial Study Group (1987) Effects of enalapril on mortality in severe congestive heart failure. results of the Cooperative North Scandinavian Enalapril Survival Study (CONSENSUS). *N Engl J Med* 316: 1429–1435.
- Turnbull, F., Neal, B., Ninomiya, T., Algert, C., Arima, H., Barzi, F. *et al.* (2008) Effects of different regimens to lower blood pressure on major cardiovascular events in older and younger adults: meta-analysis of randomised trials. *Br Med J* 336: 1121–1123.
- Unger, T. (2002) The role of the renin–angiotensin system in the development of cardiovascular disease. *Am J Cardiol* 89: 3A–9A; discussion 10A.
- Verdecchia, P., Schillaci, G., Borgioni, C., Ciucci, A., Gattobigio, R., Zampi, I. *et al.* (1998) Prognostic significance of serial changes in left ventricular mass in essential hypertension. *Circulation* 97: 48–54.
- Whaley-Connell, A., Habibi, J., Rehmer, N., Ardhani, S., Hayden, M., Pulakat, L. *et al.* (2013) Renin inhibition and AT1R blockade improve metabolic signaling, oxidant stress and myocardial tissue remodeling. *Metabolism* 62: 861–872.
- Yusuf, S., Hawken, S., Ôunpuu, S., Dans, T., Avezum, A., Lanas, F. *et al.* (2004) Effect of potentially modifiable risk factors associated with myocardial infarction in 52 countries (the Interheart Study): case-control study. *Lancet* 364: 937–952.
- Yusuf, S., Sleight, P., Pogue, J., Bosch, J., Davies, R. and Dagenais, G. (2000) Effects of an angiotensin-converting-enzyme inhibitor, ramipril, on cardiovascular events in high-risk patients. *Engl J Med* 342: 145–153.
- Zablocki, D. and Sadoshima, J. (2010) The one-two punch: knocking out angiotensin II in the heart. *J Clin Invest* 120: 1028–1031.
- Zheng, J., Wei, C., Hase, N., Shi, K., Killingsworth, C., Litovsky, S. *et al.* (2014) Chymase Mediates injury and mitochondrial damage in cardiomyocytes during acute ischemia/reperfusion in the dog. *PLoS One* 9: e94732.
- Zhou, P., Cheng, C., Li, T., Ferrario, C. and Cheng, H. (2015) Modulation of cardiac L-type Ca²⁺ current by angiotensin-(1-7): normal versus heart failure. *Ther Adv Cardiovasc Dis* 9: 342–353.

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