Combination renin–angiotensin system blockade and angiotensin-converting enzyme 2 in experimental myocardial infarction: implications for future therapeutic directions


*Department of Medicine, University of Melbourne, Austin Health, Heidelberg, Victoria, Australia, †Peter Munk Cardiac Centre, Toronto General Hospital, Toronto, Canada, and ‡Department of Cardiology, Austin Health, Heidelberg, Victoria, Australia

ABSTRACT

The RAS (renin–angiotensin system) is activated after MI (myocardial infarction), and RAS blockade with ACEIs [ACE (angiotensin-converting enzyme) inhibitors] or ARBs (angiotensin receptor blockers) slows but does not completely prevent progression to heart failure. Cardiac ACE is increased after MI and leads to the formation of the vasoconstrictor AngII (angiotensin II). The enzyme ACE2 is also activated after MI and degrades AngII to generate the vasodilator Ang-(1–7) [angiotensin-(1–7)]. Overexpression of ACE2 offers cardioprotective effects in experimental MI, but there is conflicting evidence as to whether the benefits of ACEIs and ARBs are mediated through increasing ACE2 after MI. In the present study, we assessed the effect of an ACEi and ARB, alone and in combination, on cardiac ACE2 in a rat MI model. MI rats received vehicle, ACEi (ramipril; 1 mg/kg of body weight), ARB (valsartan; 10 mg/kg of body weight) or combination (ramipril at 1 mg/kg of body weight and valsartan at 10 mg/kg of body weight) orally for 28 days. Sham-operated rats were also studied and received vehicle alone. MI increased LV (left ventricular) mass (P < 0.0001), impaired cardiac contractility (P < 0.05) and activated cardiac ACE2 with increased gene (P < 0.05) and protein expression (viable myocardium, P < 0.05; border zone, P < 0.001; infarct, P < 0.05). Ramipril and valsartan improved remodelling (P < 0.05), with no additional effect of dual therapy. Although ramipril inhibited ACE, and valsartan blocked the angiotensin receptor, neither treatment alone nor in combination augmented cardiac ACE2 expression. These results suggest that the cardioprotective effects of ramipril and valsartan are not mediated through up-regulation of cardiac ACE2. Strategies that do augment ACE2 after MI may be a useful addition to standard RAS blockade after MI.

INTRODUCTION

Activation of the RAS (renin–angiotensin system) plays a critical role in the pathophysiology of MI (myocardial infarction), and the development of heart failure [1]. RAS blockade with ACEIs [ACE (angiotensin-converting enzyme) inhibitors] or ARBs (angiotensin receptor blockers) improves cardiac remodelling and outcomes.

Key words: angiotensin-converting enzyme, angiotensin-converting enzyme inhibitor, angiotensin receptor blocker, myocardial infarction, renin–angiotensin system.

Abbreviations: AngI etc., angiotensin I etc.; Ang-(1–7), angiotensin-(1–7); ACE, angiotensin-converting enzyme; ACEi, ACE inhibitor; ARB, angiotensin receptor blocker; AT1, AngII type 1; BP, blood pressure; BNP, brain natriuretic peptide; CVD, cardiovascular disease; FAM, 6-carboxyfluorescein; HR, heart rate; LV, left ventricular; MAP, mean arterial pressure; MI, myocardial infarction; qRT–PCR, quantitative real-time PCR; RAS, renin–angiotensin system; SHR, spontaneously hypertensive rat; TAMRA, 6-carboxytetramethylrhodamine.

Correspondence: Professor Louise Burrell (email l.burrell@unimelb.edu.au).
in both experimental models of MI as well as in man [2–4]. ACE converts AngI (angiotensin I) into the vasoconstrictor AngII, which is thought to be responsible for most of the pathophysiological effects of the RAS. Although the RAS is the major vasoconstrictor hormone system under investigation in CVD (cardiovascular disease), a counterbalancing vasodilatory arm is now known to exist [5]. The discovery of the enzyme, ACE2 which is expressed mainly in the heart and kidney [6], is of major importance given the role of ACE2 to cleave AngII to the vasodilator and anti-fibrotic peptide Ang-(1–7) [7,8], which acts through the G-protein-coupled Ang-(1–7) or mas receptor [9].

ACE2 is now recognized as an important negative regulator of the RAS and its expression in tissues is a major determinant of injury. After experimental MI, there is significant activation of cardiac ACE2 [10], which acts to combat the adverse effects of an activated cardiac RAS. Other evidence for a cardioprotective role for ACE2 arises from studies in ACE2-knockout mice, where the loss of ACE2 facilitates adverse post-MI ventricular remodelling, presumably because of removal of a degradative pathway for AngII [11], and studies showing that ACE2 overexpression in MI rats improves cardiac contractility and remodelling [12,13].

The effect of RAS blockade with ACEis [10,14] or ARBs [15] on cardiac ACE2 in post-MI rats has produced variable results. We have reported no further increase in cardiac ACE2 with ACEis in the MI rat beyond that due to MI itself [10], but others have shown that ARBs can increase ACE2 in the post-MI heart [15]. However, in the latter study [15], MI itself did not increase either ACE or ACE2, suggesting that the effect of RAS blockade on ACE2 may be strain and/or model specific.

To date, there have been no studies to assess both ACEis and ARBs in a rat model of MI, and none that have assessed the effect of dual therapy on ACE2 post-MI. As RAS blockade post-MI is standard therapy and slows but does not prevent the progression of cardiac dysfunction, we hypothesized that this may relate to a lack of increase in cardiac ACE2, beyond that which occurs as a result of MI. If this was the case, then strategies that led to augmentation of cardiac ACE2 either through gene transfer [12,13] or with ACE2 activators [16] may be novel therapeutic approaches that could be used in addition to standard RAS blockade in the future.

The aim of the present study was to examine the effect of the ACEi ramipril and the ARB valsartan alone or in combination on expression of cardiac ACE2 gene and protein in rats with MI. The effect of treatment on BP (blood pressure), and cardiac remodelling and LV (left ventricular) function was also assessed.

MATERIALS AND METHODS

Ethics and animals

Experimental procedures were performed in accordance with the National Health and Medical Research Council of Australia guidelines for animal experimentation and were approved by the Animal Ethics Committee, Austin Health. Female Sprague–Dawley rats (150–200 g) were housed in a 12 h light/12 h dark cycle with ad libitum access to rat chow containing 0.4–0.6 % NaCl (Norco) and tap water.

Surgical production of MI

Rats were intubated and ventilated with a mixture of oxygen and isoflurane (Abbott). LV free wall MI was induced by ligation of the left coronary artery as previously described [10,17,18]. Sham-operated (Control) rats underwent the same surgical procedure but the suture was not tied. Buprenorphine (Reckitt Benckiser Healthcare) analgesia was administered (0.15 μg/kg of body weight, subcutaneous) for 2 days post-operatively.

Study protocol

All rats operated on to produce an MI, and those that were alive 24 h later were randomized to the following treatment by oral gavage for 28 days: vehicle (water), ramipril (1 mg/kg of body weight per day), valsartan (10 mg/kg of body weight per day) and ramipril + valsartan in the same doses as used alone. Control rats received vehicle only.

On day 28, rats were anaesthetized (intraperitoneally sodium pentobarbitone, 60 mg/kg of body weight), and cardiac haemodynamics determined using a microtipped pressure transducer catheter (1.5F; Millar) inserted into the left carotid artery and advanced into the LV [19]. Data were analysed using Millar conductance data acquisition and analysis software. HR (heart rate), MAP (mean arterial pressure), maximum rate of isovolumic pressure development (+dP/dt\text{max}) and decay (−dP/dt\text{min}) and the time constant of isovolumic relaxation (Tau) were measured.

Rats were then killed by a lethal dose of sodium pentobarbitone, decapitated and trunk blood collected in tubes containing heparin (15 units/ml of blood) for measurement of ACE and ACE2 activity, and in tubes containing an endopeptidase inhibitor mix [20 μl/ml blood; 50 mmol EDTA, 0.2 NEM (N-ethylmaleimide) and 1–2 TIU (trypsin inhibitor unit)/ml aprotinin]. The heart was removed, weighed and the ventricles separated from the atria. The LV/interventricular septum was dissected, weighed, a thin transverse slice removed from the midline of the infarct and fixed in 10 % buffered formalin and paraaffin embedded. The remaining LV pieces were frozen at −80°C for in vitro autoradiography and quantitative real-time reverse transcriptase–PCR.
Plasma ACE, ACE2, AngII and Ang-(1–7)

Plasma ACE activity was measured using an enzymatic assay [19], where 5 μl of plasma was incubated at 37 °C with the ACE substrate hippuryl-His-Leu (1 mM) in the presence and absence of EDTA (10 mM) for 30 min. The rate of substrate cleaved was determined by comparison with a standard curve of the product His-Leu and results are expressed as nmol of substrate/ml/h.

Plasma ACE2 activity was measured using an enzymatic assay as described previously [19,20]. Plasma was incubated with an ACE2-specific QFS [quenched enzymatic assay as described previously [19,20]]. Plasma ACE2 activity was assayed with 10 μM Z-Pro-prolinal (Auspep), with or without 100 μM EDTA, in a total volume of 200 μl. The rate of substrate cleavage was determined by comparison with a standard curve of the product His-Leu and results are expressed as nmol of substrate/ml per h.

Measurement of infarct size

LV paraffin sections (4 μm) were stained with Picosirius Red (0.1% solution in saturated aqueous picric acid) (Sigma–Aldrich), and infarct size determined by measuring the ratio of scar length to ventricular circumference for each epicardial and endocardial surface. Infarct size is expressed as a percentage of the total ventricular circumference [10,18].

Immunohistochemistry for ACE2 protein

Immunohistochemical staining for cardiac ACE2 (polyclonal antibody T17; Santa Cruz Biotechnology; diluted 1:100) was performed in control (n = 26) and MI (vehicle, n = 29; ramipril, n = 19; valsartan, n = 21; and ramipril + valsartan, n = 13) rats, as described previously [10]. Sections (4 μm) were rehydrated, blocked with 3% (v/v) H2O2 and 10% (v/v) normal goat serum before incubation with primary antibody at 4 °C overnight. The secondary antibody was goat anti-rabbit at a dilution of 1:400. Antibody labelling was visualized using an ABC (avidin–biotin complex kit; Vector Laboratories) and DAB (diaminobenzidine) before counterstaining with haematoxylin/eosin. Negative control sections were incubated in the absence of primary antibody. Images were acquired on an Olympus BX50 microscope (objective lens, ×20/0.50), using a Leica DFC480 camera (Leica) and the density of ACE2 staining was quantified using computerized image analysis (AIS Imaging). In MI hearts, three areas were quantified: the viable myocardium, the infarct and the border zone. The results are expressed as a percentage of binding in the LV of control rats.

qRT-PCR (quantitative real-time-PCR)

Rat MI hearts were divided into two parts: viable myocardium and border/infarct zone, and RNA was isolated from both areas (n = 7–10 per group) using the RNASE kit (Qiagen). cDNA was synthesized with a reverse transcriptase reaction using standard techniques (Superscript II kit; Life Technologies) as described previously [10]. All primers and probes were designed using the software program ‘Primer Express’ (PE Applied Biosystems) and obtained from Geneworks.

For the ACE gene, the primers and probe were as follows: forward primer, 5′-CACCAGCAAGGTCTGT-CTT; reverse primer, 5′-CTTGCGCTAGTTTCGT-GAGAAA; and probe, FAM (6-carboxyfluorescein)-5′-CAATAGACTGCACTCTGTT-CCC-3′-TA-MRA (6-carboxytetramethylrhodamine). For the ACE2 gene: forward primer, 5′-GCCAGAGATGACCGG-AAA; reverse primer, 5′-CTGAAGTCTCCATGTCC-CAGATC; and probe, FAM-5′-TTGTCTGCCACC-CACA-3′-TAMRA. For the rat AT1 receptor gene: forward primer, 5′-CGGCTTCGGATAACATGA; reverse primer, 5′-CTCTGGATCTCCGATGTCC-GACAG; and probe, FAM-5′-ATGGATATGAGAGAGGAAATACCTC-GTT-3′-TAMRA. For the mas receptor gene: forward primer, 5′-ATGTTGGACCACCCCAT-CTGTTT-3′-TAMRA. For the rat BNP (brain natriuretic peptide) gene: forward primer, 5′-GGCGCTGGAGAGGTCAC; reverse primer, 5′-AGCTTCTGATCGTGAATGT;
and probe, FAM-5’-TCCTAGCCAGTCTTC-3’-TAMRA. qRT–PCR was performed using a multiplex method with 18S RNA (Invitrogen) as the endogenous control. The relative expression method was used to assess changes in gene expression levels, using the control group as the calibrator, which was given a value of 1.

**Statistical analysis**

Results are presented as means ± S.E.M. $P$ values were calculated using an unpaired Student’s $t$ test when comparing control and MI-vehicle and ANOVA followed by post-hoc Bonferroni analysis when comparing MI-vehicle with MI-treatment groups. For analysis of circulating peptides, results were log-transformed to stabilize variance before analysis. $P < 0.05$ was considered significant.

**RESULTS**

Cages were examined twice daily to assess the health of the animals. No control animal had evidence of cardiac damage. The mortality of the MI surgery was 36% with most deaths occurring within the first 24 h. Only rats operated upon to produce an MI and those that were alive at 24 h were randomly allocated to treatment groups. Results are reported on control ($n = 26$), MI-vehicle ($n = 29$), MI-ramipril ($n = 19$), MI-valsartan ($n = 21$) and MI-ramipril + valsartan ($n = 13$). The uneven group size in the MI rats has arisen as not all rats operated on to produce an MI had an infarct and were therefore excluded. Also only rats with an infarct size of $>20\%$ and $<60\%$ were included.

**Cardiac weight and haemodynamics**

Table 1 shows the organ weight and cardiac function after MI and the effect of intervention. There was no difference in body weight or HR between MI and control rats. MI rats had an average infarct size of 38%, which was not affected by treatment. MI resulted in adverse cardiac remodelling with increased LV mass ($P < 0.001$) and systolic dysfunction ($dP/dt_{\text{max}}; P < 0.05$) and diastolic dysfunction ($dP/dt_{\text{min}}; P < 0.001$, Tau; $P < 0.01$). The ACEi and ARB alone or in combination significantly lowered systolic and diastolic BP and MAP (all $P < 0.01$) and improved cardiac remodelling with a reduction in both the heart weight and LV corrected for body weight (all $P < 0.05$). The ACEi and ARB alone or in combination had no effect on LV function.

**Plasma RAS**

Table 2 shows the circulating components of the RAS after MI and the effect of treatment. Plasma ACE was not increased in MI rats ($P = 0.30$), and ACEi alone and in combination significantly reduced ACE activity ($P < 0.001$), as did AT$_1$ receptor blockade albeit to a lesser degree ($P < 0.05$). Plasma ACE2 activity increased following MI ($P < 0.05$) and was reduced in rats treated with ramipril ($P < 0.01$), and valsartan ($P < 0.05$), but not the combination. MI did not change plasma AngII but ramipril and valsartan significantly increased plasma AngII ($P < 0.05$), although not when used in combination. This lack of significance may have resulted from the variability in plasma levels, as well as the smaller numbers in the combination group. Plasma Ang-(1–7) was significantly increased with ramipril and valsartan ($P < 0.001$) alone, and in combination ($P < 0.01$).
Cardiac BNP gene expression

Cardiac BNP mRNA was increased in both viable myocardium and the border/infarct zone (P < 0.01; Table 3) after MI (P < 0.05). Ramipril reduced cardiac BNP mRNA in the border/scar region, alone (P < 0.05) and in combination with valsartan (P < 0.05). Only combination therapy reduced BNP mRNA in the viable myocardium (P < 0.05).

Cardiac ACE gene and protein expression

A 3-fold increase in cardiac ACE mRNA expression was observed in the border/infarct zone after MI (P < 0.05; Table 3), but was unchanged in the viable myocardium. Treatment did not affect ACE mRNA in any region of the infarcted heart.

The change in ACE protein paralleled the changes at gene level, with increased ACE binding in the border/infarct zone (P < 0.001; Figure 1B), but no change in the viable myocardium (Figure 1A). Ramipril significantly inhibited cardiac ACE activity in both areas of the heart (Figures 1A and 1B) when given alone or in combination therapy. Valsartan had no effect of ACE binding in the infarct zone, but was associated with reduced ACE activity in the viable myocardium (P < 0.01).

Cardiac AT₁ receptor gene and protein expression

Cardiac AT₁ receptor mRNA was up-regulated in the border/infarct zone after MI (P < 0.001; Table 3), and unchanged in the viable myocardium compared with control rats. No treatment affected AT₁ mRNA expression in the viable myocardium or border/infarct zone.

There was a marked increase in AT₁ receptor binding in the border/infarct zone after MI (P < 0.001; Figure 1D),
Cardiac ACE2 gene and protein expression
Cardiac ACE2 mRNA expression increased in the border/infarct zone \((P < 0.05; \text{Table 3})\), compared with control myocardium. No significant change was seen in the viable myocardium. Drug treatment had no effect on ACE2 mRNA expression compared with MI alone.

Protein levels of ACE2 in MI hearts confirmed the changes seen at the gene level, with increased ACE2 protein expression in the infarct \((P < 0.05; \text{Figure 2C})\) and border zone \((P < 0.001; \text{Figure 2B})\). ACE2 protein expression was also increased in the viable myocardium \((P < 0.05; \text{Figure 2A})\). Neither ramipril nor valsartan, alone or in combination, led to further changes in ACE2 protein expression, compared with MI-Vehicle.

Cardiac mas receptor gene expression
Cardiac mas receptor mRNA was up-regulated in the border/infarct zone after MI \((P < 0.05, \text{Table 3})\), and unchanged in the viable myocardium. No treatment affected mas receptor mRNA in the infarcted heart.

DISCUSSION
The present study is the first to assess the effect of both an ACEi and ARB, alone and in combination, on cardiac ACE2 gene and protein expression in an experimental model of MI. This model, originally described by Pfeffer and Braunwald [2], is associated with marked activation of cardiac ACE [10,23], a key factor in the progression of cardiac disease [1]. We reported previously that MI
activated both cardiac ACE and ACE2 [10], and that ramipril inhibited cardiac ACE but did not lead to further increases in cardiac ACE2, despite beneficial effects on cardiac remodelling. In the present paper, we confirm that MI is associated with activation of cardiac ACE and ACE2, and show that the ACEi and ARB alone or in combination led to further increases in cardiac ACE2 gene and protein expression over that caused by MI. In addition, RAS blockade with ACEis and ARBs was associated with increased plasma AngII, as well as significant increases in plasma Ang-(1–7).

The increase in cardiac and plasma ACE2 activity noted after experimental MI is consistent with studies in patients with heart failure, where both cardiac ACE2 gene [24] and protein are increased [10], and where plasma ACE2 activity correlates with heart failure severity [25] and clinical outcomes [26]. We have recently reported that increased plasma ACE2 activity is associated with adverse cardiac outcomes in post-operative patients [27]. There may also be a temporal change in ACE2 activity after cardiac injury. Others have shown that plasma and cardiac ACE2 increased in rats 1 week after MI, but, at 8 weeks, neither cardiac nor plasma ACE2 were elevated [14]. Whether the long-term lack of increase is a cause or consequence of ongoing LV dysfunction is not clear. We have, however, reported a similar effect in rats with chronic kidney disease; cardiac ACE2 was increased at one week post subtotal nephrectomy [20] but at 4 weeks when renal failure was established, cardiac ACE2 deficiency occurred and was thought to contribute to the progression of cardiac injury in this model [28]. In the present study, we also show that plasma ACE2 activity may be modulated by therapy with RAS blockade; although ACE2 is not directly inhibited by ACEis [6], the improvement in cardiac remodelling by therapy may in turn affect circulating levels of ACE2.

With regard to the circulating angiotensin peptides, we report significant increases in plasma AngII not only with valsartan, but also with an ACEi. The present study also shows that plasma AngII levels were not significantly increased with combination treatment, although the study is limited by the uneven group size of the MI rats. It has been shown previously in both rats and in humans that AngII can ‘escape’ from the effect of ACE inhibition [29], resulting in either no change in circulating AngII levels [30] or increased AngII levels, possibly due to AngII generation via ACE-independent pathways [31]. Indeed, this was the rationale behind the clinical trials combining ACEi with ARB post-MI and in heart failure. We also report significant increases in plasma Ang-(1–7) with both ACEis and ARBs. ACE is one of the degradative pathways for circulating Ang-(1–7) [32,33], and ACE inhibition is known to increase plasma Ang-(1–7) [34]. ARBs can increase Ang-(1–7) through increased formation from AngI, and by AngII degradation [35]. The increase in plasma Ang-(1–7) may contribute to the BP-lowering effects of RAS blockade, and preventing the increase in plasma Ang-(1–7) can reduce the antihypertensive action of ACE inhibition [36]. Increased Ang-(1–7) may also have direct cardioprotective effects, as infusion of Ang-(1–7) improved cardiac function in ischaemia/reperfusion injury [37] and preserved cardiac function and coronary perfusion in the rat MI model [38]. The present study is the first to assess the mas receptor in the infarcted rat heart and, interestingly, we saw a significant increase in mas receptor gene expression in the border/infarct zone post-MI, which remained increased even in the presence of high circulating Ang-(1–7) levels. This suggests that the increase in Ang-(1–7) may have physiological benefits post-MI. Further studies in which RAS blockade is combined with a blocker of the Ang-(1–7) or mas receptor would be needed to confirm or refute this suggestion.

There are variable results in the literature with regard to the changes in cardiac ACE2 levels and the effect of RAS blockade on cardiac ACE2 in experimental MI. There have been studies of ACEis [14] and ARBs [15], but not yet the combination in the MI model, and results of intervention may depend on the effect of MI itself on cardiac ACE2, as well as the rat strain. Thus there is no change in ACE2 mRNA in Lewis rats post-MI [15], but there is a marked increase in cardiac mRNA and protein post-MI in Sprague–Dawley rats [10,14]. In wild-type mice, MI was associated with decreased ACE2 mRNA but increased ACE2 protein [11]. In the present study, the change in ACE2 mRNA and protein paralleled each other in the border/infarct zone, but increased ACE2 protein in the viable myocardium was not associated with a change in gene expression. It has been suggested that the uncoupling of cardiac ACE2 mRNA and protein after MI may relate to post-transcriptional regulation involving the TACE [TNFα (tumour necrosis factor α-converting enzyme)] or ADAM (a disintegrin and metalloproteinase)-17 family [39].

With regard to treatment, the ARBs losartan and olmesartan increase cardiac ACE2 mRNA post-MI in Lewis rats [15], but in this strain there is no ACE or ACE2 activation as a result of MI alone. As activation of the RAS is pivotal for the development of heart failure, the Lewis rat may not be the ideal strain to study the effects of MI. With regard to the effect of dual RAS blockade on cardiac ACE2, there has only been one other study, which was in the normotensive Lewis rat, with no cardiac disease [34]. The authors reported that the ACEi lisinopril increased cardiac ACE2 mRNA but not ACE2 activity, the ARB losartan increased both gene and ACE2 activity and dual ARB/ACEi treatment had no additive effect on ACE2 activity [34].

As AngII may be synthesized through non-ACE mediated pathways [31], dual ACEi/ARB was thought to be a more effective strategy to further reduce cardiovascular morbidity and mortality. In the present study,
both ramipril and valsartan partially ameliorated cardiac remodelling, but neither ACEi nor ARB alone or in combination improved cardiac function despite potent cardiac ACE inhibitor and AT1-receptor-blocking effects. These results are in keeping with human studies of dual ACEi/ARB therapy in patients with MI [4]; VALIANT (Valsartan in Acute Myocardial Infarction Trial) showed that valsartan and captopril had equivalent effects to reduce primary end points post-MI, but the combination had no added benefits to improve outcomes [4].

We have suggested previously that increased cardiac ACE2 after MI [10] may act as a counter regulatory mechanism to limit the adverse effects of elevated cardiac AngII. As ACE2 both increases the degradation of AngII and leads to the formation of Ang-(1–7), the alteration of the relative balance of vasoconstrictor and vasodilatory Ang peptides has important consequences within the heart. Our results indicate that the beneficial cardiac effects of valsartan and/or ramipril occur independently of any change in cardiac ACE2, and lead us to speculate that strategies that would augment cardiac ACE2 activity may be a useful therapeutic strategy after MI to preserve cardiac function and prevent the development of heart failure.

A variety of approaches have been used to up-regulate ACE2 in the experimental setting including ACE2 gene transfer [12,13], all-trans retinoic acid [40] and small-molecule ACE2 activators [16]. In the SHR (spontaneously hypertensive rat) [41], ACE2 overexpression with intra-cardiac lentiviral ACE2 gene transfer reduced BP, cardiac hypertrophy and cardiac fibrosis. In MI rats, myocardial lentiviral ACE2 gene transfer shortly before coronary artery ligation preserved cardiac function and cardiac contractility and attenuated LV wall thinning [12]. Similar results occurred when adenoviral ACE2 gene transfer occurred 2 weeks after MI in rats, and led to reduced cardiac fibrosis and LV remodelling, and improved systolic function [13]. The discovery of small molecule ACE2 activators, such as xanthenone, which reversed renal [16] and cardiac fibrosis [42] in SHR, and human recombinant ACE2, which reduced the progression of diabetic nephopathy and AngII-dependent renal injury [43], are exciting developments in the field.

To date, there have been no reports on the combination of RAS blockade with either ACE2 overexpression or an ACE2 activator. Such studies are needed to test the hypothesis that augmentation of ACE2 over and above that due to cardiac injury itself would have further beneficial effects. Although there is great interest in this novel approach to CVD [35,44], further long-term studies are needed in experimental disease models as ACE2 overexpression may not always be beneficial. In ACE2 transgenic mice, there was a high incidence of sudden death that correlated with transgene expression levels [45], and sustained overexpression of ACE2 in the heart of stroke-prone SHRs led to severe fibrosis and impaired cardiac function [46].

In summary, cardiac ACE2 is increased after experimental MI, and the beneficial effects of RAS blockade on cardiac remodelling with ramipril, valsartan or combination were similar and occurred with no further increase in cardiac ACE2 expression. As overexpression of cardiac ACE2 offers cardioprotective advantages in experimental models, we speculate that strategies that do augment cardiac ACE2 after MI may be a potential adjunctive therapy to standard RAS blockade for the treatment of ischaemic heart disease. Further studies are needed that combine either ACE2 overexpression or ACE2 activators with standard RAS blockade to test this hypothesis.

**CLINICAL PERSPECTIVES**

- There is now abundant evidence for a counter-regulatory axis within the RAS in which the pathological effects of ACE are ameliorated by ACE2 and its ability to degrade AngII to Ang-(1–7), thus shifting the relative balance of vasoconstrictors and vasodilators within the cardiovascular system.
- In the present study, experimental MI was associated with activation of cardiac ACE and ACE2; ACE inhibition with ramipril and angiotensin receptor blockade with valsartan improved cardiac remodelling, but no intervention, either alone or in combination, led to further increases in cardiac ACE2 beyond that due to MI itself.
- Overexpression of ACE2 in experimental models leads to cardiac benefits, but to date there have been no reports on the combination of ACE2 overexpression and RAS blockade. Such studies are needed to test the hypothesis that augmentation of ACE2 over and above that due to cardiac injury itself would have further beneficial effects to preserve cardiac function and halt the progression to heart failure.

**AUTHOR CONTRIBUTION**

Louise Burrell conceived the experiments and co-wrote the paper. Luke Burchill, Elena Velkoska, Rachael Dean, Karen Griggs and Sheila Patel carried out various aspects of the study. Luke Burchill and Elena Velkoska analysed the data and co-wrote the paper. Sheila Patel and Rachael Dean co-wrote the paper.

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