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Myocardial infarction increases ACE2 expression in rat and humans

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KEYWORDS
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Aims Angiotensin converting enzyme (ACE) 2 catalyses the cleavage of angiotensin (Ang) I to Ang 1-9 and of Ang II to Ang 1-7. ACE2 deficiency impairs cardiac contractility and upregulates hypoxia-induced genes, suggesting a link with myocardial ischaemia. We studied the expression of ACE2 after myocardial infarction (MI) in the rat as well as in human failing hearts.
Methods and results Rats were killed at days 1, 3, and 28 after MI, or treated for 4 weeks with the ACE inhibitor ramipril (1 mg/kg). Cardiac gene and protein expression of ACE and ACE2 were assessed by quantitative real-time reverse transcriptase–polymerase chain reaction and immunohistochemistry/activity assays/*in vitro* autoradiography, respectively. Both ACE ($P = 0.022$) and ACE2 ($P = 0.015$) mRNA increased in the border/infarct area compared with the viable area at day 3 after MI. By day 28, increases in ACE ($P = 0.005$) and ACE2 ($P = 0.006$) mRNA were also seen in the viable myocardium of MI rats compared with myocardium of control rats. ACE2 protein localized to macrophages, vascular endothelium, smooth muscle, and myocytes. Ramipril attenuated cardiac hypertrophy and inhibited cardiac ACE. In contrast, ramipril had no effect on cardiac ACE2 mRNA, which remained elevated in all areas of the MI rat heart. Immunoreactivity of both ACE and ACE2 increased in failing human hearts.
Conclusion The increase in ACE2 after MI suggests that it plays an important role in the negative modulation of the renin angiotensin system in the generation and degradation of angiotensin peptides after cardiac injury.

Introduction

Angiotensin converting enzyme (ACE), a dipeptidyl carboxypeptidase, is a key enzyme in the renin angiotensin aldosterone system (RAAS) and converts angiotensin (Ang) I to the vasoconstrictor Ang II.[1,2] ACE2 is a homologue of ACE, and its amino-terminal domain has 42% homology with ACE.[3,4] ACE2 has one active enzymatic site and cleaves a single residue from Ang I to generate Ang 1-9 and degrades Ang II to the vasodilator Ang 1-7.[5,6] ACE2 expression is limited to the heart, kidney, and testis and its *in vitro* activity is unaffected by ACE inhibitors.[3,4]

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The role of ACE2 remains to be determined, but its localization in the heart suggests an important role for cardiovascular function. Rats with hypertension have low ACE2 protein levels, whereas ACE2 knockout mice have severe impairment of cardiac contractility with upregulation of hypoxia-induced genes in the heart suggesting a link to myocardial ischaemia.[7] Significant activation of the cardiac RAAS occurs after myocardial infarction (MI),[8–10] but whether this extends to changes in ACE2 is unknown. To explore this possibility, we examined the temporal and regional expression of cardiac ACE2 and ACE mRNA and protein after MI, as well as the effect of treatment with the ACE inhibitor, ramipril. We also examined ACE2 expression in explanted failing hearts from heart transplant recipients.

Methods

Experimental procedures were approved by the Austin Health Research Ethics Committee and performed according to the National Health and Medical Research Council of Australia Guidelines for animal experimentation and the principles of the Helsinki declaration for human tissue. Rats were housed in a 12:12 h light/dark cycle, with *ad libitum* food containing 0.4–0.6% NaCl (Norco) and water.

Surgical production of MI

Left ventricular (LV) free wall MI was induced in Sprague–Dawley rats (150–200 g) by ligation of the left coronary artery.[11–13] Rats were allocated to sham operation in a random manner. These rats (control) underwent the same surgical procedure as the infarct rats but the suture was not tied. Only rats that survived 24 h post-operatively were included in Studies 1 and 2. In rats operated on to produce an infarct ~80% were alive at 24 h and were then randomized to different groups. Rats with an infarct size of <20% were excluded from analysis.

Study 1: temporal and spatial expression of ACE and ACE2 following MI

Control and MI rats that survived 24 h post-operatively were randomized into groups and killed on day 1, 3, or 28. In all hearts, the LV/interventricular septum was dissected and weighed; a thin transverse slice was removed from the midline and fixed in 10% buffered formalin and paraffin embedded. Sections were stained both with Masson's trichrome for assessment of infarct size and with haematoxylin and eosin. The mean epicardial and endocardial scar circumference was compared with total LV circumference to calculate total infarct size.[11] The remaining LV pieces were frozen at −80°C for *in vitro* autoradiography (IVA) and quantitative real-time reverse transcriptase–polymerase chain reaction (QRT–PCR), which were performed on a randomly selected subset of hearts.

Study 2: effect of ACE inhibition on ACE and ACE2 after MI in the rat

MI rats were randomized to oral ramipril (1 mg/kg) or vehicle for 28 days. Sham-operated rats (control) were also studied and received only vehicle. All rats were killed on day 28. The LV was dissected, weighed, and infarct size determined. The remaining LV pieces were frozen for assessment of ACE and ACE2 mRNA by QRT–PCR, and ACE and ACE2 by IVA and activity assay, respectively.

Study 3: immunolocalization of ACE2 in human heart

The LVs of explanted failing hearts from five male patients with ischaemic heart disease (IHD) who had undergone cardiac transplantation were assessed. Subjects were aged 49 ± 2 years and had severe coronary atheroma with 86 ± 4% stenosis of the left anterior descending coronary artery and 80 ± 8% stenosis of the right coronary artery. All were on standard anti-heart failure medication including ACE inhibitors, beta-blockers, and diuretics. Control LV tissue ($n = 3$) was obtained at post-mortem from male patients (age 49, 72, and 74 years) with no cardiac history and no pathological abnormalities of the heart. Tissues were fixed in 10% buffered formalin and paraffin embedded for immunohistochemical localization of ACE and ACE2.

Extraction of total RNA, synthesis of cDNA, and QRT—PCR

Rat MI hearts were divided into two parts: viable myocardium and infarct/border zone, and RNA was isolated from both areas. For control rats, the whole LV was used. Total RNA was isolated using the RNeasy kit method (Qiagen). cDNA was synthesized with a reverse transcriptase reaction using standard techniques (Superscript II kit, Life Technologies, Gaithersburg, MD, USA) as previously described.[14,15] QRT–PCR is a fully quantitative method for the determination of amounts of mRNA. Briefly, gene-specific 5′-oligonucleotide corresponding to rat ACE (5′-CACCGGCAAGGTCTGCTT), ACE 3′-oligonucleotide primer (5′-CTTGGCATAGTTTCGTGAGGAA), and ACE probe (FAM5′-CAACAAGACTGCCACCTGCTGGTCC-TAMRA); for ACE2 gene-specific 5′-oligonucleotide corresponding to (5′-ACCCTTC TTACATCAGCCCTACTG), an ACE2 3′-oligonucleotide primer (5′-TGTCCAAAACCTACCCCACATAT), and ACE2 probe (FAM5′-ATG CCTCCCTGCTCATTTGCTTGGT-TAMRA) were designed using the software program 'Primer Express' (PE Applied Biosystems, Foster City, CA, USA). QRT–PCR was carried out using a multiplex method with 18S VIC as the endogenous control.[14] A relative expression method was applied in this study using the control group as the calibrator. The calculations for fold induction are extrapolated as follows. The cycle number (Ct) for 18S was subtracted from the Ct of the gene of interest (in this case ACE or ACE2), resulting in the difference in Ct, that is, DCt. The average DCt was then calculated for the calibrator group and this value was subtracted from every DCt value in all groups, resulting in a DDCt value for all samples. The DDCt value was then entered into the equation $2(\exp - \mathrm{DDCt})$, which resulted in a fold induction value. All groups were compared with the calibrator group (which has a value of 1).

Cardiac ACE activity

In Study 2, rat cardiac ACE ($n = 5$ per group) was assessed by IVA on LV sections (20 μm) using the specific radioligand ^{125}I-MK351A ($K_i = 30$ pmol/L).[12] Quantification of four sections from each rat was performed using a microcomputer-imaging device (Imaging Research, Ontario, Canada). In MI hearts, the viable myocardium, infarct, and border zone were quantified separately.[13] The border zone is the area of high cellular infiltrate at the border zone of the scar tissue of the infarct. The optical density of the autoradiographs was calibrated in terms of radioactivity density (d.p.m./mm^2).

Cardiac ACE2 activity

In Study 2, rat MI hearts ($n = 5$–8 per group) were divided into viable myocardium and infarct/border zone, and membranes prepared from each area. The whole LV was used for control rats ($n = 8$). Tissue was homogenized in ice-cold Tris-buffered saline (TBS: 25 mM Tris–HCl, 125 mM NaCl, pH 7.4), and homogenates pelleted by ultracentrifugation at 100 000*g* for 60 min at 4°C, resuspended in fresh TBS, and rehomogenized. Following a second ultracentrifugation step, the final membrane pellet was resuspended in 0.5 mL TBS, aliquoted, and frozen at −70°C. Prior to ACE2 activity assay, an aliquot of each sample was thawed on ice, diluted 10-fold in ACE2 assay buffer (100 mM Tris, 1 M NaCl, pH 6.5), and briefly sonicated (Branson Cell Disruptor B-30) in order to disperse the membranes. Protein was determined using a Bio-Rad DC Protein Assay kit.

Quenched fluorescent substrate assay of ACE2 activity

Cardiac membrane preparations (100 μL diluted sample) were incubated in triplicate with an ACE2-specific quenched fluorescent substrate (QFS), (7-methoxycoumarin-4-yl)-acetyl-Ala-Pro-Lys (2,4-dintirophenyl) (Auspep, Parkville, Victoria, Australia) as previously published.[5] Assays were performed with 50 μM QFS in a final volume of 200 μL ACE2 assay buffer (100 mM Tris, 1 M NaCl, pH 6.5). The final concentration of dimethylsulfoxide (used to solubilize QFS) was 0.7%. Reactions proceeded at 37°C for 60 min within a thermostatted *f*Max fluorescence microplate reader (Molecular Devices, Sunnyvale, CA, USA), prior to reading the liberated fluorescence ($\lambda_{ex} =$ 320 nm, $\lambda_{em} = 420$ nm). As the QFS can be cleaved by prolyl endopeptidase, a specific inhibitor of this enzyme, Z-Pro-prolinal (1 μM), was included in all wells.[16] To further confirm the specificity of the assay, the measurement was repeated in the presence of the ACE2 inhibitor C16.[17] The specific activity of the preparations was expressed as units of fluorescence per milligram of membrane protein per hour.

ACE and ACE2 immunohistochemistry

Immunohistochemical staining for ACE2 (polyclonal antibody from Millennium Pharmaceuticals Inc., Cambridge, MA, USA) and ACE (human polyclonal ACE antibody[18]) were performed in rat heart (ACE2) and human heart (ACE and ACE2) as outlined subsequently. Both antibodies were used at a dilution of 1:500. The specificity of the ACE2 antibody has been shown in heart and kidney[3] and that of ACE antibody in human vessels.[18] Immunohistochemistry for macrophages was carried out as previously described.[13]

Immunohistochemistry was carried out on 4 μm sections prepared from paraffin embedded LV of human hearts as well as MI and control rat hearts. Sections were dewaxed and hydrated, then endogenous peroxidase activity quenched for 20 min incubation in 3% H_2O_2 in distilled water. The primary antibodies were applied for 1 h at room temperature. The Elite Vectastain ABC kit (Vector Laboratories, Burlingame, CA, USA) followed by diaminobenzidine (Sigma, St Louis, MO, USA) were used to visualize antibody binding. Slides were counterstained with haematoxylin and cover-slipped. Negative control slides were incubated with normal goat serum; the primary antibody was excluded.

Statistics

Data are presented as mean ± SEM. The sample size was chosen on the basis that six to eight rats are sufficient to show infarct sizes of >20% compared with 0% in sham-operated rats. Significant differences were obtained when $P < 0.05$, and all *P*-values were calculated from two-tailed tests of statistical significance. Data were analysed where appropriate using ANOVA and the Fisher least significant difference test for multiple comparisons. In addition, the myocardium of control rats was compared with the viable myocardium of MI rats using unpaired *t*-tests. Within MI hearts, the different areas of the heart (viable, infarct/border) were compared using paired *t*-tests. In Study 2, in MI rats the effect of ramipril was compared with vehicle in the different regions of the heart using unpaired *t*-tests. For QRT–PCR, values for the controls were arbitrarily standardized to 1 by taking the average of the results of all control hearts, and the data for MI rats expressed relative to this value.

Results

Cages were examined twice daily to assess the health of the animals. No control animal had evidence of cardiac damage.

Study 1: temporal and spatial expression of ACE and ACE2 mRNA

Of 69 rats operated on to produce an MI, 81% ($n = 56$) were alive at 24 h and were randomized to control day 1 ($n = 9$), post-MI day 1 ($n = 10$), control day 3 ($n = 9$), post-MI day 3 ($n = 10$), control day 28 ($n = 10$), and post-MI day 28 ($n = 9$). Four rats died during the course of Study 1 and were excluded from further analysis, as were rats with an MI of <20%. Hence, results are reported on 18 rats (post-MI day 1, $n = 8$; post-MI day 3, $n = 4$; post-MI day 28, $n = 6$) and 27 control rats ($n = 9$ per time point). Infarct size could not be determined at day 1 post-MI but was 49 ± 4% at day 3 and 38 ± 4% at day 28. Total heart mass and LV mass increased in MI compared with control rats at all time points ($P < 0.001$, data not shown).

ACE and ACE2 mRNA expression

Figure 1 demonstrates the relative quantification of cardiac ACE and ACE2 mRNA, which was assessed in a subset of rat hearts and increased after MI in a temporal and spatial manner. There were nine control rats at each time point and four to eight MI rats at each time point. At day 3, ACE mRNA was elevated in the border/infarct zone [6.7 ± 1.3 arbitrary units (AU)] compared with the MI-viable area (2.7 ± 1.1 AU) ($P = 0.022$), and also increased in the MI-viable myocardium by day 28 (2.4 ± 0.3 AU) compared with control rats (1.08 ± 0.2 AU) ($P = 0.005$). Similar changes were noted in the temporal and spatial expression of ACE2 mRNA after MI. Significant increases in ACE2 expression were seen in the border/infarct zone of MI rats at day 3 (4.38 ± 0.7 AU) compared with the MI-viable area (1.8 ± 0.3 AU) ($P = 0.015$), and these changes persisted at day 28 (3.0 ± 0.5 AU). In addition, at day 28, ACE2 mRNA was elevated three-fold in the MI-viable myocardium (2.87 ± 0.4 AU) compared with control myocardium (1.17 ± 0.2 AU) ($P = 0.006$).

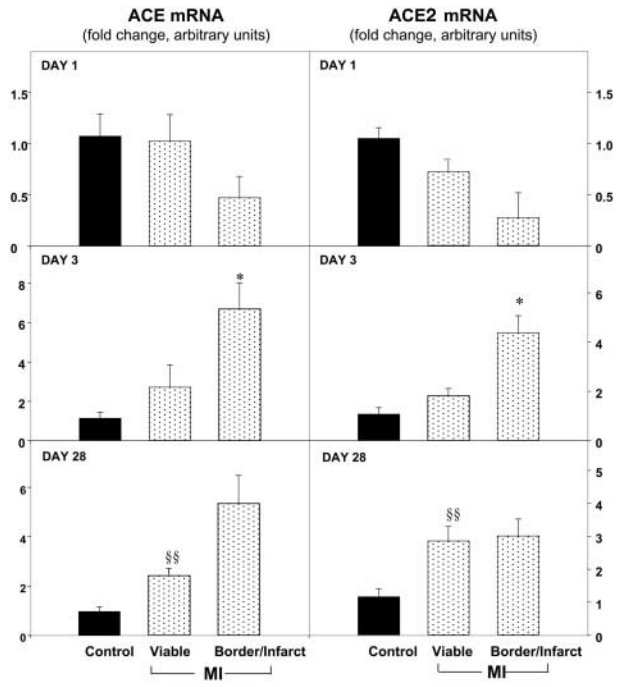

Figure 1 Quantification of ACE and ACE2 mRNA levels in hearts from control and MI rats measured by QRT–PCR. Rat MI hearts were divided into two parts: viable myocardium and infarct/border zone, and RNA isolated from these two areas separately. Data are shown as mean ± SEM. For control, $n = 9$ per time point and for MI, day 1, $n = 8$; day 3, $n = 4$; day 28, $n = 6$). ACE mRNA: §§$P = 0.005$, MI-viable vs. control; *$P = 0.022$, MI-viable vs. border/infarct zone. ACE2 mRNA: §§$P = 0.006$, MI-viable vs. control; *$P = 0.015$ MI-viable vs. border/infarct zone.

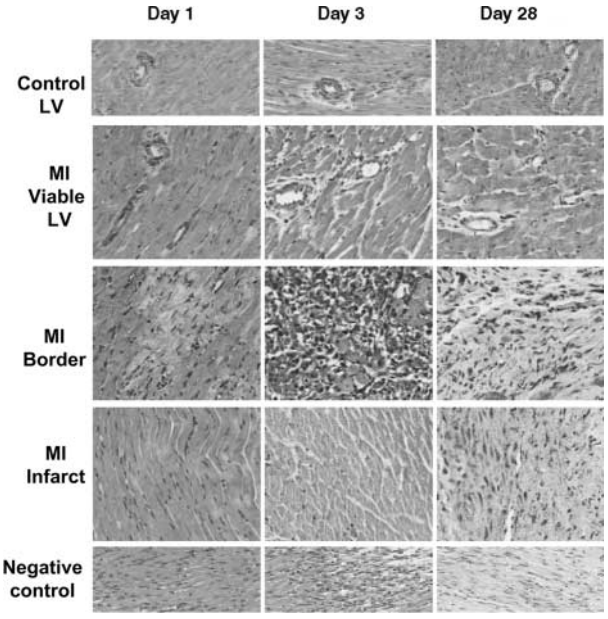

Figure 2 Immunohistochemical staining of ACE2 protein (brown colour—see online supplementary material for the colour version of this figure) in control hearts (upper panel) and hearts after MI. Staining is shown in the viable myocardium, border area, and infarct of LV on days 1, 3, and 28 post-MI. The cardiac myocyte cell membranes of MI rats were stained with ACE2 protein as were the infiltrating cells and vessels in both the infarct and border areas. Immunostaining was increased after MI in all areas of the LV but was especially increased in infiltrating cells of the border zone at day 3. Negative control slides were incubated with normal goat serum and the primary antibody was excluded; the results in the lower panels show no immunostaining (×200).

Expression of ACE2 protein by immunohistochemistry

Figure 2 demonstrates the qualitative immunohistochemical localization of ACE2 in the heart. ACE2 protein was present in viable myocardium, border zone, and the infarct, and was localized to the endothelium of small to large arteries as well as sporadically within the smooth muscle of these vessels, in addition to being associated with myocytes. ACE2 immunoreactivity was especially increased in the border zone of the MI at 3 days, and specifically, ACE2 immunoreactivity was strongly associated with infiltrating mononuclear inflammatory cells identified morphologically as macrophages (*Figure 2*). By 28 days following MI, the inflammatory response in the border zone had subsided and a well-developed infarct was present with foci of immunoreactivity for ACE2 found within macrophages.

Study 2: effect of ACE inhibition on ACE and ACE2 after MI in the rat

As with Study 1, ~80% of MI rats were alive at 24 h and were randomized to vehicle or ramipril. There were no deaths during this study. The sham-operated rats (control, $n = 8$) had no evidence of infarction and there was no difference in MI size in rats treated with vehicle ($n = 6$, 42 ± 2%) or ramipril ($n = 8$, 38 ± 3%). Ramipril significantly lowered systolic blood pressure at week 4 in MI rats (control, 142 ± 6; MI-vehicle, 140 ± 6; MI-ramipril 110 ± 6 mmHg, $P = 0.01$). LV mass increased after MI (control 2.4 ± 0.01; MI-vehicle, 2.9 ± 0.01 g/kg BW, $P < 0.001$), and there was regression of LV hypertrophy with ramipril with a reduction in LV mass (2.5 ± 0.01 g/kg BW, $P < 0.001$ vs. MI-vehicle).

ACE and ACE2 mRNA expression

ACE and ACE2 mRNA expression was assessed in a subset of rat hearts ($n = 4$–6 per group), and *Figure 3* shows the quantification of mRNA expression. At day 28 post-MI, both ACE ($P = 0.005$) and ACE2 ($P = 0.041$) mRNA increased in the viable myocardium compared with control myocardium, confirming the results of Study 1. There were further increases in ACE mRNA in the border/infarct zone of MI rats compared with MI-viable myocardium ($P = 0.049$). In MI rats, ACE inhibition decreased ACE mRNA in the viable myocardium ($P = 0.014$) and the border/infarct zone ($P = 0.003$) compared with the vehicle treatment. In contrast, ramipril had no effect on cardiac ACE2 mRNA, which remained elevated in both the viable and the border/infarct zone of the MI rat heart.

ACE and ACE2 activity

Protein levels of ACE in control and MI rats confirmed the changes seen at the gene level (*Figure 4*) with significant

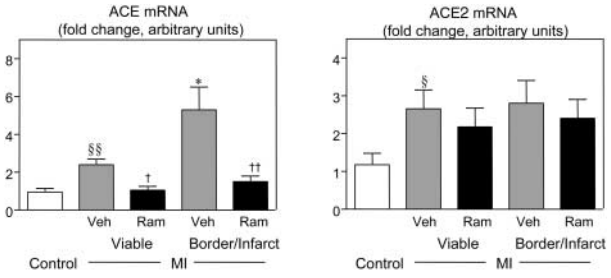

Figure 3 Quantification of ACE and ACE2 mRNA levels in hearts from control and MI rats measured by QRT–PCR. Rat MI hearts were divided into two parts: viable myocardium and border/infarct zone, and RNA was isolated from these two areas separately. Data are shown as mean ± SEM; $n = 6$ per group for control, $n = 4$–6 per group for MI. ACE mRNA: §§$P = 0.005$, MI-viable vs. control; *$P = 0.049$ MI-viable vs. border/infarct zone; †$P = 0.014$, ††$P = 0.003$, MI-vehicle vs. MI-ramipril. ACE2 mRNA: §$P = 0.041$, MI-viable vs. control.

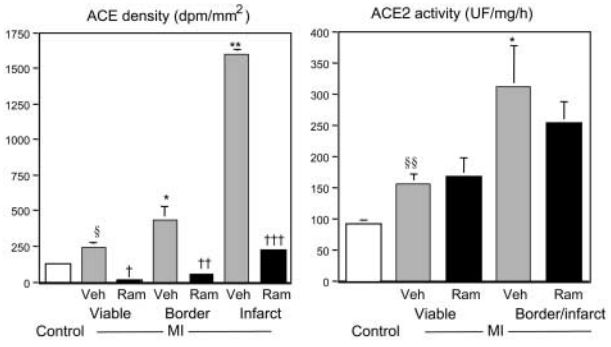

Figure 4 Quantification of ^{125}I-351A binding to cardiac ACE in control and MI rats treated with vehicle or the ACE inhibitor ramipril. Cardiac ACE has been quantified separately in infarct, border, and viable myocardium. Data are shown as mean ± SEM; $n = 5$ per group. §$P = 0.018$, MI-viable vs. control; *$P = 0.015$, MI-viable vs. border; **$P = 0.0014$, MI-viable vs. infarct; †$P = 0.009$, ††$P = 0.003$, †††$P < 0.0001$, MI-vehicle vs. MI-ramipril. The right panel shows cardiac ACE2 activity in the viable myocardium and the border/infarct zone in control and MI rats treated with vehicle or ramipril. Data are shown as mean ± SEM; $n = 8$ per group for control and MI-ramipril, $n = 5$–6 per group for MI-vehicle. §§$P = 0.002$, MI-viable vs. control; *$P = 0.027$, MI-viable vs. border/infarct.

increases in ACE in the infarct ($P = 0.0014$) and border zone ($P = 0.015$) compared with the viable myocardium of MI rats. In addition, ACE increased in the viable myocardium of MI rats compared with control rats ($P = 0.018$). Treatment with ramipril significantly inhibited cardiac ACE activity in the viable myocardium ($P = 0.009$), the border zone ($P = 0.003$), and the infarct zone ($P < 0.0001$) of MI rats. ACE2 activity was significantly increased in the viable myocardium of MI-vehicle rats compared with control rats ($P = 0.002$), and increased further in the border/infarct zone ($P = 0.027$). In those MI rats receiving ACE inhibition with ramipril, cardiac ACE2 activity remained significantly increased.

Study 3: immunolocalization of ACE2 in human heart

Figure 5 demonstrates the qualitative immunohistochemical localization of ACE and ACE2 in a non-ischaemic human LV and a ventricle from a patient with ischaemic heart disease. Both ACE and ACE2 immunoreactivity were qualitatively increased in the cardiac tissue of all five patients with failing hearts due to ischaemic heart disease compared with the two non-ischaemic controls. ACE and ACE2 immunoreactivity was predominately localized to vascular endothelium and smooth muscle and was also seen in cardiomyocytes.

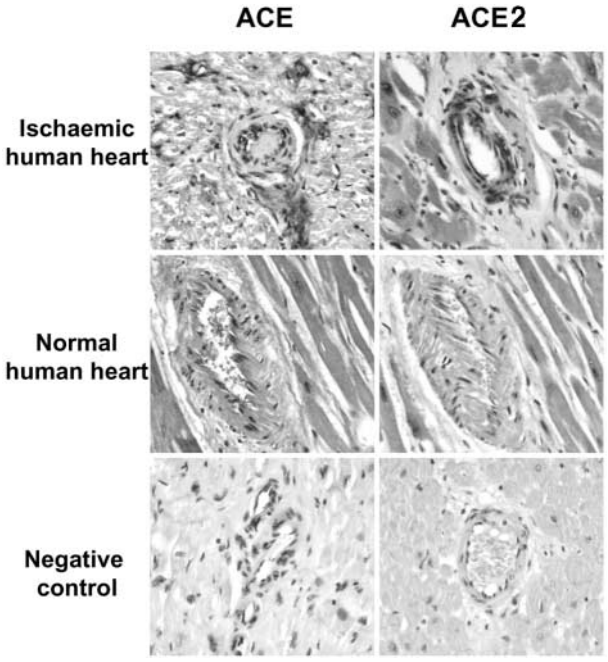

Figure 5 Immunohistochemical staining of ACE and ACE2 protein (brown colour—see online supplementary material for the colour version of this figure) in normal and failing human cardiac tissue. Negative control slides were incubated with normal goat serum and the primary antibody was excluded; there was no immunostaining (×200).

Discussion

The major finding of this study is that MI results in increased expression of the novel peptidase ACE2 in both rat and humans. In the rat, we found marked myocardial expression of ACE2 in the injured tissue (infarct and border zone) early after MI, with further increases occurring in the viable myocardium by 28 days. ACE2 protein expression was also increased in the ischaemic cardiac explants of heart transplant recipients. ACE2 expression co-localized with that of ACE in both human and rat tissue.

Components of the RAAS, such as ACE and Ang II are activated after MI[8,19–22] and the finding that ACE2 increases in a similar spatial and temporal manner to ACE supports a regulated response to injury and mechanical stress. ACE2 is also highly expressed by infiltrating mononuclear cells, suggesting that it may participate in the initial inflammatory response to injury. As ACE2 immunoreactivity was also observed in endothelial cells and myocytes some time after MI in both rat and humans, ACE2 may also be involved in the late phase of post-MI changes when injury and inflammation have

abated but mechanical load remains high and the non-infarcted myocardium is undergoing a complex series of molecular and cellular events that lead to changes in the shape and function of the myocardium.[23]

The benefits of ACE inhibition to improve cardiac function and morbidity and mortality after MI[24] are due in part to reduction in cardiac ACE and Ang II levels.[20,25,26] Indeed, the present study confirmed that ACE inhibition was associated with reduced LV mass. Cardiac ACE was inhibited by ramipril but ACE2, as assessed by a range of techniques including specific assessment of ACE2 catalytic activity, was unchanged, which is consistent with the *in vitro* data, which have previously demonstrated that ACE inhibitors do not inhibit ACE2 activity.[4]

To date, there have been no studies on ACE2 in the context of MI. Increased cardiac ACE2 after MI may act as a counter-regulatory mechanism to limit the adverse effects of an elevated cardiac Ang II by increasing levels of the vasodilator Ang 1-7. It is possible that the relative balance of vasoconstrictor and vasodilatory angiotensin peptides is important in the modulation of both haemodynamic and trophic effects of these peptides within the heart. Support for this idea comes from studies in ACE2 knockout mice,[7] which have severely impaired cardiac contractility in the setting of elevated Ang II levels and can be rescued by simultaneous genetic ablation of ACE. Furthermore, studies in the MI rat have shown that the development of heart failure is associated with increased cardiac Ang 1-7 immunoreactivity[27] and that infusion of Ang 1-7 attenuates the development of heart failure after MI.[28] It is possible that the relative balance of vasoconstrictor and vasodilator angiotensin peptides is important in the modulation of both haemodynamic and trophic effects of these peptides in the context of MI. Finally, the localization of ACE2 in rat and human heart to endothelial cells of intra-myocardial blood vessels and smooth muscle cells also supports a role for ACE2 in the control of local vasodilation.

It has been reported previously that ACE2 protein expression was similar in non-failing human hearts and the failing heart from one patient with idiopathic cardiomyopathy.[3] However, a recent report examined 14 subjects with idiopathic cardiomyopathy and showed an increase in functional cardiac ACE2 activity as assessed by the *ex vivo* formation of Ang 1-7.[29] The results of the present study extend the findings of increased cardiac ACE2 activity to the context of ischaemic heart disease.

The finding that hypoxia-induced gene expression is upregulated in ACE2-deficient mice may provide an explanation for changes in ACE2 in ischaemic myocardium. Although there have been no other studies on the expression of ACE2 after tissue injury, ACE2 expression is reduced in experimental hypertension[7] and diabetes,[14] while expression of collectrin, a protein with significant homology to ACE2, is upregulated in a model of progressive renal injury.[30]

Perspectives

ACE2 is a newly described enzyme identified in rodents and humans. The importance of ACE2 both in normal physiology and in various pathophysiological states is largely unknown. ACE2 has a more restricted distribution than ACE, being expressed mainly in the heart and kidney. ACE2 knockout studies have illustrated the potential physiological role of ACE2 in cardiac function, and this study identifies a pathological disorder, MI, which is associated with increased cardiac ACE2 expression in rat and humans. Further examination of the impact of ACE2 on the generation of angiotensin and kinin peptides in cardiovascular disorders is required. Further clarification of the role of ACE2 in disease will be assisted by the development of agents which can modulate ACE2 activity. It is anticipated that ACE2 will be a target for the development of therapeutics which could be relevant to heart failure.

Supplementary material

Supplementary material is available at *European Heart Journal* online.

Acknowledgements

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