Role of the ACE2/Angiotensin 1–7 Axis of the Renin–Angiotensin System in Heart Failure

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Abstract: Heart failure (HF) remains the most common cause of death and disability, and a major economic burden, in industrialized nations. Physiological, pharmacological, and clinical studies have demonstrated that activation of the renin–angiotensin system is a key mediator of HF progression. Angiotensin-converting enzyme 2 (ACE2), a homolog of ACE, is a monocarboxypeptidase that converts angiotensin II into angiotensin 1–7 (Ang 1–7) which, by virtue of its actions on the Mas receptor, opposes the molecular and cellular effects of angiotensin II. ACE2 is widely expressed in cardiomyocytes, cardiofibroblasts, and coronary endothelial cells. Recent preclinical translational studies confirmed a critical counter-regulatory role of ACE2/Ang 1–7 axis on the activated renin–angiotensin system that results in HF with preserved ejection fraction. Although loss of ACE2 enhances susceptibility to HF, increasing ACE2 levels prevent and reverse the HF phenotype. ACE2 and Ang 1–7 have emerged as a key protective pathway against HF with reduced and preserved ejection fraction. Recombinant human ACE2 has been tested in phase I and II clinical trials without adverse effects while lowering and increasing plasma angiotensin II and Ang 1–7 levels, respectively. This review discusses the transcriptional and post-transcriptional regulation of ACE2 and the role of the ACE2/Ang 1–7 axis in cardiac physiology and in the pathophysiology of HF. The pharmacological and therapeutic potential of enhancing ACE2/Ang 1–7 action as a novel therapy for HF is highlighted. (Circ Res. 2016;118:1313-1326. DOI: 10.1161/CIRCRESAHA.116.307708.)

Key Words: angiotensin II ■ angiotensin 1–7 ■ angiotensin-converting enzyme 2 ■ heart failure ■ renin-angiotensin system

The renin–angiotensin system (RAS) is a peptidergic system that functions in the homeostatic control of the cardiovascular and renal systems and in regulating extracellular fluid volume. Inhibition of the RAS plays a central role in alleviating the increased morbidity and mortality of patients with heart failure (HF).1,2 The RAS consists of a series of enzymatic reactions that result in generation of angiotensin II (Ang II). In the first step, renin (an aspartyl proteinase secreted by kidney into the circulation) cleaves hepatic peptide angiotensinogen to produce Ang I in the blood. Ang I is then hydrolyzed by angiotensin-converting enzyme (ACE) in the second step, producing the octapeptide Ang II. This biologically active peptide acts on Ang II type 1 and type 2 receptors (AT$_1$R and AT$_2$R; Figure 1A). Ang II promotes vasoconstriction, inflammation, salt and water reabsorption, and oxidative stress via the activation of AT$_1$R.3 These detrimental effects of Ang II/AT$_1$R have encouraged the quest for a counter-regulatory axis of the activated RAS. RAS was initially thought to function as a systemic entity not localized to any specific tissue. However, this notion of systemic RAS was challenged by observations that many tissues are capable of synthesizing the key components of RAS, including heart, kidney, vasculature, pancreas, retina, and others. The local RAS could produce peptides at...
ACE2: Discovery, Biochemistry, and Regulation

Discovery of ACE2 and Its Differences From ACE

ACE2 or ACE homolog was discovered as a zinc metalloproteinase by 2 different groups in 2000. ACE2 was initially identified from human HF and lymphoma cDNA libraries and was later shown to serve as a receptor for the severe acute respiratory syndrome coronavirus. It was found to possess an apparent signal peptide, a transmembrane domain, and a single metalloproteinase active site containing an HEXXH zinc-binding domain. ACE2 is a type I transmembrane protein with an extracellular N-terminal domain containing the catalytic site and an intracellular C-terminal tail. Similar to ACE, the catalytic site of ACE2 is exposed (an ectoenzyme) to circulating vasoactive peptides. Expression of a soluble truncated form of ACE2 in Chinese hamster ovary cells produced a glycoprotein of 120 kDa that was able to cleave Ang I and II but not bradykinin. Other critical residues typical of the ACE family are conserved in ACE2. Tipnis et al discovered that the ACE2 gene contains 18 exons, with several having considerable size similar to the first 17 exons of human ACE. The metalloproteinase catalytic domains of ACE2 and ACE are 42% identical according to the findings of Donoghue et al. In spite of such similarity though, unlike ACE, ACE2 does not convert Ang I to Ang II. In fact, ACE2 activity is inhibited by EDTA but is unaffected by ACE inhibitors (ACEis), such as captopril and lisinopril. Further research revealed a major difference in enzymatic actions of ACE and ACE2. ACE acts a dipeptidyl carboxypeptidase (removing a dipeptide from the C terminus of substrate), whereas ACE2 acts as a monocarboxypeptidase (removing a single amino acid) that degrades Ang I to generate the nonapeptide Ang 1–9 and Ang II to generate the heptapeptide Ang 1–7. Later studies focused on ACE2 purification and characterization of its catalytic activity, showing a pH optimum of 6.5 and enhancement of ACE2 activity by monovalent anions, including Cl− and F−, but not Br−. This is consistent with the activity of ACE. However, ACE2 was later shown to possess one Cl− binding site compared with 2 Cl− sites in ACE. Of 126 biological peptides tested with ACE2 using liquid chromatography–mass spectrometry, ACE2 hydrolyzed 3 peptides with high efficiency: Ang II, apelin-13, and dynorphin A 1 to 13. ACE2 also showed a preference for cleaving C-terminal amino acids with peptides ending in Pro-X, where X is a hydrophobic amino acid. This cleavage preference of ACE2 was supported by a key experiment in which a dipeptide, Pro-Phe, completely inhibited ACE2 activity at 180 μmol/L with Ang II as the substrate. In a search for the active site residues of ACE2, site-directed mutagenesis revealed that Arg273 is critical for substrate binding and its replacement causes complete loss of enzyme activity.

The difference in ACE and ACE2 enzymatic activity became more evident on the discovery that human ACE2 catalytic efficiency is 400-fold higher with Ang II as a substrate than with Ang I. To further unravel the biological role and importance of ACE2, several ACE2 inhibitors were designed and synthesized via substrate-based pharmacophore design and virtual screening. MLN-4760, a potent and selective inhibitor developed with substrate-based...
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Proteolytic Processing, Transcriptional, and Post-Transcriptional Regulation of ACE2

Various molecules are shed from cell surfaces by the action of a disintegrin and metalloproteinase (ADAM) 17, also known as tumor necrosis factor-α-converting enzyme. ADAM17-mediated proteolysis of ACE2 releases an enzymatically active ectodomain from the cell surface, generating a soluble, active form of the enzyme. Lambert et al. confirmed the ectodomain shedding of heterologously expressed ACE2 in HEK293 cells and endogenously expressed ACE2 in Huh7 cells. Small interfering RNA against ADAM17 reduced the shedding of ACE2 and ADAM17 overexpression increased it, providing direct evidence of ADAM17-mediated ectodomain shedding of ACE2. Lambert et al. later discovered that calmodulin, a ubiquitous calcium-binding protein, associates with ACE2 and prevents its shedding, an action inhibited by calmodulin inhibitors. However, increased ACE2 shedding mediated by calmodulin inhibitors was only partially blocked by metalloproteinase inhibitor, suggesting the involvement of alternate proteolytic pathways not yet identified. The initial observation of ACE2 shedding was further confirmed and shown to be a constitutive and regulated phenomenon in various cell types, including Chinese hamster ovary cells, fibroblasts, 3T3-L1 adipocytes, neurons, cardiomyocytes, and proximal tubular cells. In particular, we identified a positive feedback mechanism in the RAS whereby Ang II facilitates the loss of candidate ACE2 substrates, including Ang I and Ang 1-7. This positive feedback loop involves the activation of the RAS and the shedding of ACE2, which in turn activates the RAS and further enhances ACE2 shedding. This cycle is essential for the pathogenesis of heart failure and other cardiovascular diseases.

Figure 1. Enzymatic cascade of the renin–angiotensin system (RAS), key receptor systems, and the biological effects mediated by angiotensin II (Ang II) and Ang 1–7. A. The RAS cascade showing the angiotensin peptide metabolic pathway. Angiotensinogen, as the starting substrate, is cleaved by renin to Ang I. Ang I is cleaved by angiotensin-converting enzyme (ACE) to Ang II, which is cleaved by ACE2 to Ang 1–7. Ang II acts on AT1 receptors. Ang 1–7 acts on Mas receptors and counterbalances the Ang II type 1 receptor (AT1) actions. B. Decreased ACE2 shifts the balance in the RAS to the Ang II/AT1 axis, resulting in disease progression. Increased ACE2 (by rhACE2, gene delivery, or ACE2 activators) shifts the balance to the Ang 1–7/MasR axis, leading to protection from disease. APA indicates aminopeptidase A; PCP, prolyl carboxypeptidase; and rhACE2, recombinant human ACE2.
its negative regulator, ACE2. Ang II action on AT₁R leads to phosphorylation (mediated by p38 mitogen-activated protein kinase) and activation of ADAM17, resulting in increased ACE2 shedding (Figure 2). Shedding of membrane-bound ACE2 is likely responsible for the loss of myocardial ACE2 and elevation in plasma ACE2 activity in HF that correlates with worsened prognosis. The biological and clinical significance of ACE2 ectodomain shedding is yet to be fully characterized. The inhibition of ectodomain shedding of ACE2 by manipulating the enzyme activity of ADAM17 could have therapeutic potential in HF.

A reporter system using the 3′-untranslated region of an ACE2 transcript was used to determine the functionality of putative microRNA-binding sites identified in vitro. In a luciferase reporter assay containing ACE2 3′-untranslated region, miR-421 strikingly decreased ACE2 protein levels, whereas loss of miR-421 reversed these effects, implying that miR-421 modulates ACE2 expression via post-translational repression rather than degradation of mRNA transcripts. This identified miR-421 as a potential regulator of ACE2 and was the first demonstration of post-transcriptional regulation of ACE2. ACE2 mRNA expression is also regulated by sirtuin 1 (SIRT1). Energy stress by hypoxia and adenosine monophosphate kinase activation by 5-amino-4-imidazolecarboxamide riboside increase the cellular ratio of nicotinamide adenine dinucleotide–oxidized form (NAD+) to nicotinamide adenine dinucleotide–reduced form (NADH) and increase ACE2 expression. SIRT1, in the presence of a possible but unknown cofactor, binds to the promoter region of ACE2 and this binding is promoted by 5-amino-4-imidazolecarboxamide riboside. 5-Amino-4-imidazolecarboxamide riboside -induced ACE2 expression is inhibited by an inhibitor of SIRT1, providing strong evidence for the SIRT1-mediated transcriptional regulation of ACE2 under conditions of energy stress (Figure 2). Similarly, apelin also increases ACE2 promoter activity in vitro and upregulates ACE2 expression in failing hearts in vivo (Figure 2). Therapeutically, agents that increase ACE2 expression (SIRT1 activators and apelin) or inhibitors of negative regulators of ACE2 (tumor necrosis factor–α-converting enzyme or miR-421) could be utilized to enhance ACE2 activity and counteract cardiovascular diseases, including HF.

**Role of ACE2/Ang 1–7 in HF**

HF is a growing epidemic with high morbidity and mortality at an international scale. Acute and chronic HF are characterized by activation of several signaling pathways associated with pathological hypertrophy and maladaptive ventricular remodeling. HF is caused by damage to or loss of myocardocytes and contributes to diminished systolic performance and diastolic dysfunction in the failing heart. HF involves changes in cardiac structure, myocardial composition, myocyte deformation, and multiple biochemical and molecular alterations, collectively referred to as adverse myocardial remodeling. Despite improvements in medical and surgical therapies, cardiac diseases remain the leading cause of death in North America, with ischemic and hypertensive heart disease as the leading cause of HF.

Diabetes mellitus and obesity are major causes of morbidity and mortality in all parts of the world, including North America. Diabetes mellitus is characterized by insulin insufficiency that is frequently associated with severe cardiovascular complications and increased risk for hypertension, HF; and
myocardial infarction (MI). 79–81 Obesity itself is an independent risk factor for development of HF with preserved ejection fraction (HF-pEF), independent of other comorbid conditions. 82–84 The rising global tide of obesity and diabetes mellitus will likely contribute further to the increasing prevalence of systolic and diastolic HF. 78,80,85–87 Although the mechanisms underlying the intertwined relationship among diabetes mellitus, obesity, hypertension, and cardiovascular events remain to be fully defined, major culprits that have been implicated are cardiovascular inflammation, oxidative stress, mitochondrial dysfunction, and insulin resistance, all closely linked with abnormalities in the RAS. 88–91

Neurohormonal changes such as activation of the RAS and increased Ang II levels play a pivotal role in adverse myocardial remodeling and progression to HF. 2,92,93 Indeed, pharmacological antagonism of the RAS using ACEi or AT1R blockers is a cornerstone of current medical therapy for human HF, including diabetic cardiomyopathy. 75–76 Although these pharmacotherapies for HF provide benefits, patients with HF continue to be plagued by clinical deterioration, high morbidity and mortality. 77,94 Irrespective of the capacity of ACEi to inhibit ACE action, Ang II levels can remain elevated in optimally treated patients with HF. About 50% of the patients using ongoing ACEi therapy exhibit elevated levels of Ang II, the result of activation of mast cell chymase. 95–98 Therefore, there is an urgent need to identify alternative strategies to minimize the detrimental effects of Ang II and treat HF.

ACE2, by virtue of its action on Ang I and Ang II, is nature’s endogenous ACEi at the cellular level (Figure 3). Ang–9, the product of ACE2 degradation of Ang I, has recently shown promising antihypertrophic, antifibrotic, and antihypertensive effects. These beneficial effects result in cardioprotection against hypertension and MI. 23–26 Adenoviral delivery of Ang–9 in H9c2 cardiomyocytes has shown antihypertrophic effects comparable with adenoviral Ang I–7 delivery. 23 Moreover, RhoA/Rho kinase inhibition has shown potent antihypertensive effects that were mediated via the upregulation of vascular and plasma ACE2 and increased plasma Ang I–9 levels, without an increase in Ang 1–7 levels. 26 This suggests a potential role for Ang I–9 in the antihypertensive effects of RhoA/Rho-kinase inhibition.

Both Ang I and Ang II can function as the preferred substrate for ACE2. Studies using recombinant human ACE2 (rhACE2) and ACE2 purified from sheep tissues showed Ang II as a preferred substrate for ACE2. 33,38,41,67,99,100 In sheep, conversion from Ang I to Ang 1–9 was not detected while the proximal tubules contained robust ACE2 activity that converted Ang II to Ang 1–7. 101 In contrast, changes in ACE2 correlated with plasma Ang I–9 levels in rats. 102 In a recent study, Ye et al demonstrated that rhACE2 generated Ang 1–7 and Ang 1–9, whereas recombinant murine ACE2 generated predominantly Ang 1–7. In addition, the therapeutic effects of rhACE2 are highly dependent on Ang 1–7 action in rodents. 30,67,100 and in human studies, rhACE2 clearly lowered plasma Ang II levels resulting in increased plasma Ang I–7 levels. 104–106 However, it remains possible that the contribution of Ang 1–9 in ACE2’s beneficial effects may be underestimated and requires further investigation with a clear emphasis on human studies.

Ang 1–7 activates MasR and exert various effects, the majority of which antagonize Ang II’s effects. 15,20 These effects include (1) activation of the phosphatidylinositol 3-kinase–Akt–endothelial nitric oxide synthase pathway, (2) inhibition of protein kinase C–p38 mitogen-activated protein kinase pathways, and (3) inhibition of collagen expression to limit cardiac fibrosis (Figure 3). 15,107,108 To understand the relative contributions of inhibiting the Ang II/AT1R axis and activating the Ang 1–7/MasR axis to cardioprotective effects, we studied the effects of irbesartan and Ang 1–7 supplementation in pressure-overload–induced HF in ACE2 knockout mice. 30 We found functional redundancy in the antifibrotic and antihypertrophic effects and suppression of pathological signaling. The cardioprotective effects of irbesartan and Ang 1–7 were equivalent, suggesting similar significance of both axes.

Role of ACE2/Ang 1–7 in Hypertension

Activated RAS and Ang II are established key mediators of hypertension; therefore, ACE2 is hypothesized to be a potent modulator of blood pressure and its deficiency leads to hypertension. In a preclinical model of hypertension, ACE2 gene maps to a defined quantitative trait locus on the X-chromosome previously identified as a quantitative locus for blood pressure. 7 Recent studies suggest an association between ACE2 activity and blood pressure levels. 109,110 Serum ACE2 activity was higher in patients with hypertension compared with healthy individuals. In hypertensive patients with type 1 diabetes mellitus, serum ACE2 activity was positively correlated with systolic blood pressure in both males and females. 110 These studies suggest that elevated ACE2 may be a compensatory response to the hypertension. Indeed, the antihypertensive role of ACE2 has also been established in various preclinical models of hypertension. 28,111–113 Lentiviral overexpression of ACE2 results in increased expression of antihypertensive components of RAS (Ang 1–7, MasR, and AT1R), attenuating the elevated blood pressure. 111,112 Similarly, rhACE2 pretreatment alleviated hypertension induced by acute Ang II infusion and was associated with decreased plasma Ang II and increased plasma Ang I–7 levels. 29,30 Cyclophilin–encapsulated Ang 1–7, AVE0091, and CGEN856S (MasR agonists) have shown blood pressure–lowering effects in hypertensive animals. 114 The antihypertensive effects of ACE2/Ang 1–7 generated interest in potential cardioprotective effects against hypertensive heart diseases, a group of disorders that includes HF, ischemic heart disease, hypertensive heart disease, and left ventricular hypertrophy.

Role of ACE2/Ang 1–7 in HF With Reduced Ejection Fraction

ACE2 plays a critical role in the control of cardiac physiology, and altered ACE2 expression or activity is linked to the progression of heart disease (Figure 1B). In heart, ACE2 is expressed in various cells including the cardiomyocytes, cardiac fibroblasts, and coronary endothelial cells, 115 where it negates Ang II actions and also activates Ang 1–7/MasR signaling (Figure 3). ACE2 expression is highly affected by pathological disease conditions, suggesting its role in counter-regulating the development of cardiac diseases. In the human
population, genetic variations in the ACE2 gene correlate with susceptibility to cardiovascular disease. Single-nucleotide polymorphisms of ACE2 are associated with variation in septal wall thickness, ventricular hypertrophy, and coronary artery disease.

The first report on the role of ACE2 as an essential regulator of cardiac function came soon after its discovery. In that study, ACE2 knockout mice showed reduced systolic function. The decrease in systolic function was both sex and time dependent, with more severe abnormalities in male than in female mice, and a more pronounced phenotype in older animals. ACE2 knockout mice also showed increased Ang II levels, which were rescued with genetic ablation of ACE7. Consistently, we found age-dependent dilated cardiomyopathy in ACE2 knockout mice. This resulted in reduced systolic function along with increased cardiac inflammation and oxidative stress. Myocardial ACE2 protein levels were decreased in pressure-overload–induced HF, suggesting an inverse relationship between myocardial ACE2 protein levels and disease progression. In addition, loss of ACE2 resulted in worsened pathological remodeling in response to pressure-overload–induced biomechanical stress. This was associated with systolic dysfunction and ventricular dilation. Both were deemed because of activation of the myocardial NADPH oxidase system, superoxide production, and matrix metalloproteinase activation, which was attributed to increased local Ang II levels.
C16, resulted in worsening of MI-induced cardiac dysfunction, increased infarct size, matrix metalloproteinase activation, cardiac extracellular matrix disruption, and inflammation (Table).123,124 Lentiviral125,126 or adenoviral127 overexpression of ACE2 ameliorated MI-induced cardiac remodeling. In addition, lentiviral infection of cultured fibroblasts decreased the acute hypoxic exposure–induced production of collagen.128 Importantly, Ang 1–7 treatment has shown noticeable cardioprotective effects in preclinical models of nonischemic and ischemic cardiomyopathy.15,21,126,129 Ang 1–7 suppressed cardiomyocyte growth in vitro and inhibited MI–induced ventricular hypertrophy in vivo. Ang 1–7 also decreased myocardial levels of proinflammatory cytokines (tumor necrosis factor-α and interleukin-6), leading to alleviation of cardiac inflammation.21,126 These results confirm the important contribution of Ang 1–7 in the cardioprotective effects of ACE2 (Figure 4).

### Table. Interventions to Modulate ACE2 Levels or Activity and Their Effects in Experimental Models of Heart Failure

<table>
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<tr>
<th>Experimental Intervention</th>
<th>Experimental Model</th>
<th>Observation</th>
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<td>Gain of function</td>
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<tr>
<td>Lentiviral overexpression</td>
<td>LAD coronary artery ligation</td>
<td>6 wk post surgery: complete rescue of cardiac output, a 41% rescue of ejection fraction, a 44% rescue in contractility, and a 53% rescue in LV anterior (infraacted) wall thinning compared with control rats125</td>
</tr>
<tr>
<td>Lentiviral overexpression</td>
<td>SHR</td>
<td>Attenuation of high blood pressure in the SHR, 18% reduction in left ventricular wall thickness, 12% increase in left ventricular end-diastolic, and a 21% increase in end-systolic diameters in lenti-ACE2–treated SHR; attenuation of perivascular fibrosis113</td>
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<tr>
<td>Lentiviral overexpression</td>
<td>Ang II infusion</td>
<td>Attenuation of the increased heart weight/body weight and myocardial fibrosis induced by Ang II infusion113</td>
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<tr>
<td>Lentiviral overexpression</td>
<td>Cardiac fibroblasts—hypoxia/reoxygenation</td>
<td>Attenuation of both basal and hypoxia/reoxygenation-induced collagen production by fibroblasts128</td>
</tr>
<tr>
<td>Adenoviral overexpression</td>
<td>LAD coronary artery ligation</td>
<td>4 wk after ACE2 gene transfer: reduced LV volume and extent of myocardial fibrosis, increased LV ejection fraction and levels of ACE2 activity127</td>
</tr>
<tr>
<td>rhACE2</td>
<td>Ang II infusion</td>
<td>Blunted the hypertrophic response and expression of hypertrophy markers; decreased ROS production; inhibited pathological signaling67; rhACE2 administration to WKY rats reduced Ang II infusion–induced pressor response, myocardial hypertrophy, pathological signaling, and superoxide production130</td>
</tr>
<tr>
<td>rhACE2</td>
<td>SHR</td>
<td>14-d administration of rHACE2 partly corrected hypertension, ROS production, and pathological signaling in the heart24</td>
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<tr>
<td>rhACE2</td>
<td>Transverse aortic constriction</td>
<td>rHACE2 partially prevented the pressure-overload–induced dilated cardiomyopathy and mRNA expression of disease markers and profibrotic genes67</td>
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<tr>
<td>ACE2 activator (DIZE)</td>
<td>LAD coronary artery ligation</td>
<td>DIZE attenuated the MI–induced decrease in fractional shortening by 89%, improved dP/dt max by 92%, and reversed ventricular hypertrophy by 18%131</td>
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<tr>
<td>Loss of function</td>
<td></td>
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<tr>
<td>ACE2KO</td>
<td>Ang II infusion</td>
<td>Worsened cardiac fibrosis and pathological hypertrophy in ACE2KO mice67</td>
</tr>
<tr>
<td>ACE2KO</td>
<td>Transverse aortic constriction</td>
<td>Eccentric cardiac remodeling, increased pathological hypertrophy, and worsening of systolic performance; increased ROS production108,118,120</td>
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<tr>
<td>ACE2KO</td>
<td>LAD coronary artery ligation</td>
<td>Enhanced susceptibility to MI, with increased mortality, infarct expansion, and adverse ventricular remodeling123</td>
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<tr>
<td>ACE2KO</td>
<td>Type 1 diabetes mellitus; Akita</td>
<td>Loss of ACE2 in type 1 diabetic mice resulted in HF–rEF with background HF–pEF in Akita mice122</td>
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<tr>
<td>ACE2KO</td>
<td>High-fat diet–induced obesity</td>
<td>Loss of ACE2 worsens epicardial adipose tissue inflammation, myocardial metabolic abnormalities, and lipotoxicity, resulting in HF–pEF130</td>
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<tr>
<td>ACE2 inhibitor (MLN4760)</td>
<td>(mRen2)27 hypertensive rats</td>
<td>Increased cardiac Ang II levels; increases in LV anterior, posterior, and relative wall thicknesses; increased interstitial collagen fraction area and cardiomyocyte hypertrophy124</td>
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<tr>
<td>ACE2 inhibitor (DX600)</td>
<td>Ang II stimulation of cultured cardiac fibroblasts</td>
<td>DX600 increased superoxide production and expression of CTGF, FKN, and phosphorylated ERK1/2; rHACE2 reduced these effects of Ang II116</td>
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<tr>
<td>ACE2 Inhibitor (C16)</td>
<td>Coronary artery ligation</td>
<td>Increase in MI size and reduction in LV % fractional shortening124</td>
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ACE2KO indicates angiotensin-converting enzyme 2 knockout; Ang II, angiotensin II; CTGF, connective tissue growth factor; DIZE, diminazene aceturate; ERK1/2, extracellular signal–regulated kinase 1/2; FKN, fractalkine; HF–rEF, heart failure with reduced ejection fraction; HF–pEF, heart failure with preserved ejection fraction; LAD, left anterior descending; LV, left ventricle; MI, myocardial infarction; rHACE2, recombinant human ACE2; ROS, reactive oxygen species; and SHR, spontaneously hypertensive rats.
It accounts for ≈30% of all patients with HF, with a similar mortality rate to patients with HF with reduced ejection fraction. Ang II–induced diastolic dysfunction is a clinically relevant, widely accepted preclinical model of HF-pEF. We and others found that loss of ACE2 resulted in worsened cardiac dysfunction, cardiac hypertrophy, and fibrosis, leading to greater diastolic dysfunction in response to Ang II (Table). Importantly, treatment with rhACE2 decreased plasma and myocardial Ang II levels and increased plasma Ang 1–7 levels, providing definitive evidence for a key role of ACE2 in the metabolism of Ang II. Furthermore, rhACE2 attenuated pathological changes mediated by Ang II, reducing myocardial hypertrophy and fibrosis and correcting diastolic dysfunction. However, treatment with rhACE2 did not affect baseline plasma Ang II, Ang 1–7, or blood pressure in wild-type mice. This suggests that substrate availability is a limiting factor in ACE2 enzymatic activity. The pursuit of molecular mechanisms for these actions identified rhACE2’s capacity to inhibit the Ang II effects on transforming growth factor-β1 activation and collagen production. Loss of ACE2 also resulted in increased production of reactive oxygen species via nicotinamide adenine dinucleotide phosphate oxidase 2 activation, which is also suppressible by rhACE2. Lentiviral overexpression of ACE2 protects the heart against myocardial injuries induced by Ang II in rats, confirming the role of ACE2 in counteracting HF-pEF. We assessed the contribution of Ang 1–7/MasR activation to the favorable effects shown by rhACE2 in the Ang II–induced murine HF model; inhibition of Ang 1–7/MasR signaling resulted in loss of rhACE2-mediated cardioprotective effects. However, this observation does not rule out the potential contribution of Ang 1–9 to the protective effects of rhACE2. An appropriate preclinical study is required to assess the relative contributions of Ang 1–9 and Ang 1–7. ACE2 is an endogenous regulator of activated RAS-induced HF-pEF and enhancing ACE2 has a marked beneficial effect.

**Role of ACE2/Ang 1–7 in Diabetes Mellitus and Obesity-Associated Cardiomyopathy**

Diabetes mellitus and obesity are major causes of morbidity and mortality in all parts of the world, including Canada. Studies of the ACE2/Ang 1–7 axis in diabetes mellitus and obesity-associated cardiac dysfunction have shed light on the critical role of this pathway in counter-regulation of the Ang II-mediated pathological changes mediated by Ang II, reducing myocardial hypertrophy and fibrosis and correcting diastolic dysfunction. However, treatment with rhACE2 did not affect baseline plasma Ang II, Ang 1–7, or blood pressure in wild-type mice. This suggests that substrate availability is a limiting factor in ACE2 enzymatic activity. The pursuit of molecular mechanisms for these actions identified rhACE2’s capacity to inhibit the Ang II effects on transforming growth factor-β1 activation and collagen production. Loss of ACE2 also resulted in increased production of reactive oxygen species via nicotinamide adenine dinucleotide phosphate oxidase 2 activation, which is also suppressible by rhACE2. Lentiviral overexpression of ACE2 protects the heart against myocardial injuries induced by Ang II in rats, confirming the role of ACE2 in counteracting HF-pEF. We assessed the contribution of Ang 1–7/MasR activation to the favorable effects shown by rhACE2 in the Ang II–induced murine HF model; inhibition of Ang 1–7/MasR signaling resulted in loss of rhACE2-mediated cardioprotective effects. However, this observation does not rule out the potential contribution of Ang 1–9 to the protective effects of rhACE2. An appropriate preclinical study is required to assess the relative contributions of Ang 1–9 and Ang 1–7. ACE2 is an endogenous regulator of activated RAS-induced HF-pEF and enhancing ACE2 has a marked beneficial effect.

**Therapeutic Approaches and Potential of Enhancing ACE2/Ang 1–7 in HF**

Irrespective of the capacity of ACEi to inhibit ACE action, Ang II levels can remain elevated in optimally treated patients with HF; ≈50% of patients using ongoing ACEi therapy exhibit elevated levels of Ang II. The generation of plasma and tissue Ang II by non–ACE-related enzymes such as chymase suggests that enhancing ACE2 action may indeed have a unique therapeutic role. In fact, ACEi and AT1R blocker have been shown to upregulate the expression of ACE2 or prevent the loss of ACE2. ADAM17–mediated ACE2 shedding represents a mechanism by which Ang II induces a positive feedback mechanism in the tissue-localized RAS, leading to its dysregulation. This results in the neurohumoral imbalance that is typical of HF. Inhibiting tumor necrosis factor-α–converting enzyme–mediated shedding of ACE2 from the surface of cardiac cells, leading to retention of ACE2 enzymatic activity within the cardiac microenvironment, might have therapeutic potential. ACE2 is post-transcriptionally regulated by miR-421, inhibition of which may result in...
increased ACE2 expression. Because ACE2 is also subject to transcriptional regulation by SIRT1 and apelin, SIRT1 activators or apelin may have therapeutic benefits by enhancing the actions of ACE2.

A well-studied tool to enhance ACE2 action is rhACE2. A randomized, double-blinded, placebo-controlled study administered intravenous rhACE2 to healthy human subjects and found that the rhACE2 was well tolerated. Despite marked changes in angiotensin system peptide concentrations, hypotension was absent, suggesting the presence of effective compensatory mechanisms in healthy volunteers.106 rhACE2 is primarily responsible for the conversion of Ang II into Ang 1–7 but can also convert Ang 1–10 into Ang 1–9.154 In healthy human volunteers treated with rhACE2, Ang II levels were reduced but Ang 1–7 levels were increased or remained unchanged.104,106 Importantly, in a recently completed phase II trial in patients with acute lung injury, rhACE2 resulted in sustained reduction in plasma Ang II levels and elevation in Ang 1–7 levels.106 We propose that assessment of plasma RAS peptide levels can allow the tailoring of rhACE2 therapy for human HF. rhACE2 provided beneficial effects against Ang II-induced HF-pEF and pressure-overload–induced HF with reduced ejection fraction in murine models of HF (Table).67 Thus, using rhACE2 as a therapy is much a viable option, and the advancement of rhACE2 in clinical trials provides the translational impact of rhACE2 findings in murine models.198,199 Several ACE2 activators and Ang 1–7/MasR agonists have been developed. In addition, novel approaches, including oral ACE2 and Ang 1–7 biencapsulated in plant cells, have been designed and used in preclinical studies, showing promising cardioprotective effects.131,155–158 Finally, gene therapy approaches could be utilized to achieve the tissue-specific delivery of ACE2/Ang 1–7.

Autologous cell-based therapy using putative progenitor cells such as CD34+ cells could be an attractive therapeutic approach for diabetic vascular complications. However, these cells are dysfunctional in diabetic individuals. Peripheral CD34+ cells isolated from patients with diabetes mellitus exhibit reduced proliferative potential and migratory function, which could be attributed to decreased endothelial nitric oxide synthase activity, increased reactive oxygen species levels, and advanced glycation end-products.159,160 Because ACE2 and Ang 1–7 are potent activators of endothelial nitric oxide synthase19 and antioxidants,100,132 the ACE2/Ang 1–7/MasR axis should improve CD34+ cell function and result in increased reparative efficacy. Indeed, Ang 1–7 increased the vascular reparative function of CD34+ cells isolated from patients with diabetes mellitus.161

Conclusions

ACE2 has emerged as the dominant mechanism for negative regulation of the RAS, by metabolizing Ang II into the beneficial peptide Ang 1–7. This important biochemical and physiological property is being harnessed as potential therapy for HF. Since the discovery of ACE2 in 2000, tremendous progress has been made in elucidating its biochemical actions and its key role in heart disease and HF. ACE2 is widely expressed and regulates the fundamental cellular biology of cardiomyocytes, cardiofibroblasts, and coronary endothelial cells in both HF with reduced ejection fraction and HF-pEF models. Ang 1–7 has also emerged in HF models as a physiologically active peptide with protective effects. Enhancing Ang 1–7 action may also provide marked therapeutic effects in HF. Clinical and experimental studies clearly support a physiological and pathophysiologic role for ACE2/Ang 1–7 in HF, and studies indicate that increasing/activating ACE2/Ang 1–7 results in beneficial effects to prevent heart disease and HF. Further experimental studies are required that combine rhACE2/ACE2 activators with RAS blockers (such as ACEi or AT1R blockers) to determine if this combined approach offers additional benefits.

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Disclosures

None.

References


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