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Drug Discovery Today: Therapeutic Strategies
Vol. 9, No. 4 2012

Heart failure

Manipulating angiotensin metabolism with angiotensin converting enzyme 2 (ACE2) in heart failure

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Angiotensin converting enzyme 2 (ACE2), is a monocarboxypeptidase which metabolizes several peptides including the degradation of Ang II, a peptide with vasoconstrictive/proliferative/effects, to generate Ang 1–7, which acting through its receptor Mas exerts vasodilatory/anti-proliferative actions. The classical pathway of the RAS involving the ACE-Ang II-AT$_1$ receptor axis is antagonized by the second arm constituted by the ACE2-Ang 1–7/Mas receptor axis. Loss of ACE2 enhances the adverse pathological remodeling susceptibility to pressure-overload and myocardial infarction. Human recombinant ACE2 is also a negative regulator of Ang II-induced myocardial hypertrophy, fibrosis and diastolic dysfunction and suppresses pressure-overload induced heart failure. Due to its characteristics, the ACE2-Ang 1–7/Mas axis may represent new possibilities for developing novel therapeutic strategies for the treatment of heart failure. Human recombinant ACE2 has been safely administered to healthy human volunteers intravenously resulting in sustained lowering of plasma Ang II levels. In this review, we will summarize the beneficial effects of ACE2 in heart disease and the potential use of human recombinant ACE2 as a novel therapy for heart failure.

Section editor:
Robert Willette – Heart Failure Drug Discovery Performance Unit, GSK Pharmaceuticals, King of Prussia, USA.

Introduction

The renin–angiotensin system (RAS) is a peptidergic system with endocrine characteristics that functions in the homeostatic control of arterial pressure, tissue perfusion, and extracellular volume. It is a central regulator of cardiovascular and renal function and plays a key role in the pathophysiology of various cardiovascular disorders [1]. The RAS consist of a series of enzymatic reactions culminating in the generation of angiotensin II (Ang II). In the first step of the enzymatic reactions, renin, an aspartyl protease secreted by kidney into the circulation, cleaves hepatic peptide angiotensinogen to produce angiotensin I (Ang I) in the blood. The second step involves hydrolysis of Ang I by angiotensin-converting enzyme (ACE), resulting in the production of the bioactive octapeptide Ang II, which promotes vasoconstriction, inflammation, salt and water reabsorption and oxidative stress [2]. The classical RAS family has seen substantial conceptual changes with

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1740-6773/$ http://dx.doi.org/10.1016/j.ddstr.2013.11.001

the identification of angiotensin-converting enzyme 2 (ACE2) as the negative regulator of the RAS. Angiotensin-converting enzyme 2 (ACE2), a homologue of the ACE, was discovered in 2000 and the functional importance of this enzyme has become unequivocal. It was initially identified from human heart failure and lymphoma cDNA libraries [3,4] and was later shown to serve as a receptor for the SARS coronavirus [5]. Both ACE and ACE2 are type-1 transmembrane enzymes with 42% homology and share identity in the cytosolic, sheddase, transmembrane and non-catalytic extracellular domains, though ACE2 is non-sensitive to ACEi [4]. In contrast to ACE, a dipeptidyl carboxypeptidase, ACE2 is a monocarboxypeptidase and degrades Ang I to generate Ang (1–9) and Ang II to generate the heptapeptide, Ang (1–7) [3,4], a biologically active metabolite. Ang (1–7) acts on the membrane bound Mas receptor (MasR), whose actions are often opposite to those attributed to Ang II [6,7]. Ang (1–7)/MasR axis has been shown to counteracting various pathways triggered by Ang II/AT_1R axis activation. These pathways include the classical hypertrophic pathways of p38 MAPK and protein kinase C (PKC) (Fig. 1). Ang II/AT_1R axis activation suppresses the PI3K/Akt pathway, whereas Ang (1–7)/MasR axis stimulates this pathway leading to increased cell survival [8]. Thus, while the ACE/Ang II/AT_1R is a well-established axis of the RAS, the ACE2/Ang (1–7)/MasR axis provides a physiological antagonism resulting in the counter-regulation of the RAS [8–11] (Fig. 1). This suggests that the effects mediated by ACE2 could be attributed to suppressed Ang II/AT_1R axis activation and/or stimulation of Ang (1–7)/MasR axis activation. To understand the relative importance of both the axes, we studied pressure overload model in ACE2 null (ACE2KO) mice and treated them with irbesartan or Ang (1–7) [12]. We found the functional redundancy in the cardioprotective effects of irbesartan as well as Ang (1–7) as shown by suppression of NADPH oxidase activity, MMP2 and MMP9 activation and pathological signaling, suggesting equivalent importance of both the axes [12]. However, cardiac and plasma RAS peptide levels assessment, from non-failing controls and patients with heart failure, is required to categorize the patients into the therapeutic categories and 'tailor' the therapy for human heart failure.

Interestingly, another important product of ACE2 mediated Ang I degradation, Ang (1–9) has recently shown promising cardioprotective effects in the animal models of hypertension and myocardial infarction [13–16]. Ang (1–9) supplementation to spontaneously hypertensive rats (SHR) did not affect the blood pressure, cardiac function or left ventricular mass index. However, Ang (1–9) significantly reduced cardiac fibrosis, the effect which was found to be mediated via AT_2R activation induced increased NO bioavailability [13]. Flores-Munoz *et al.* (2012) have studied the effects of adenoviral delivery of Ang (1–7) as well as Ang (1–9) in rat H9c2 cardiomyocytes and have found comparable anti-hypertrophic effects [16]. Recently, RhoA/Rho-kinase inhibition, a signaling pathway that participates in pathological cardiovascular and renal remodeling and also in blood pressure regulation, reduced blood pressure and increased vascular and plasma ACE2 activity along with reduced Ang II and increased Ang-(1–9) plasma levels. It can be concluded that anti-hypertensive effects of RhoA/Rho-kinase inhibition might be mediated via ACE2/Ang (1–9)/AT_2R axis as RhoA/Rho-kinase inhibition did not result in any changes in Ang-(1–7) levels. In this review, we will mainly focus on the role of ACE2 in the manipulation of Ang II metabolism, enhancing ACE2 action as a potential therapeutic regimen for heart failure and ACE2 as biomarker in heart failure.

Role of ACE2 in systolic dysfunction

Along with other members of systemic RAS, ACE2 is also widely distributed. Local RAS exists in the cardiovascular tissues, and ACE2 mRNA is present in various cell-types including the coronary microcirculation, cardiofibroblasts and cardiomyocytes [17]. ACE2 plays a critical role in the control of cardiac physiology and its altered expression is linked to major pathophysiological changes of the cardiovascular system (Figs 1,2). In the human population, genetic variability in the ACE2 gene correlates with susceptibility to cardiovascular disease [18–20]. Single nucleotides polymorphisms (SNPs) of ACE2 are associated with variation in left ventricular mass, septal wall thickness and ventricular hypertrophy [18], coronary heart disease and myocardial infarction [19] and hypertension in patients with metabolic syndrome [21]. In failing human heart ventricles there is increased Ang (1–7)-forming activity which may reflect increased ACE2 activity [22] while ACE2 gene expression is increased in dilated and ischemic cardiomyopathies [23]. Genetic targeting experiments indicate that ACE2 is an important regulator of cardiac function. The first evidence showing that ACE2 is an endogenous cardiac regulator was shown in ACE2 knockout mice where deletion of the ACE2 gene resulted in abnormal heart function [24]. Loss of ACE2 resulted in worsened pathological remodeling associated with systolic dysfunction and ventricular dilation in response to pressure overload induced biomechanical stress due to the triggered activation of the myocardial NAPDH oxidase system, superoxide production and activation of matrix metalloproteinases [12,25]. The beneficial effects of human recombinant ACE2 (hrACE2) were also demonstrated in the clinically relevant model of pressure-overload induced heart failure [26]. Interestingly, myocardial ACE2 protein levels were found to reduce substantially in wildtype mice subjected to pressure overload, thereby perpetuating the pathological effects of Ang II, suggesting an inverse

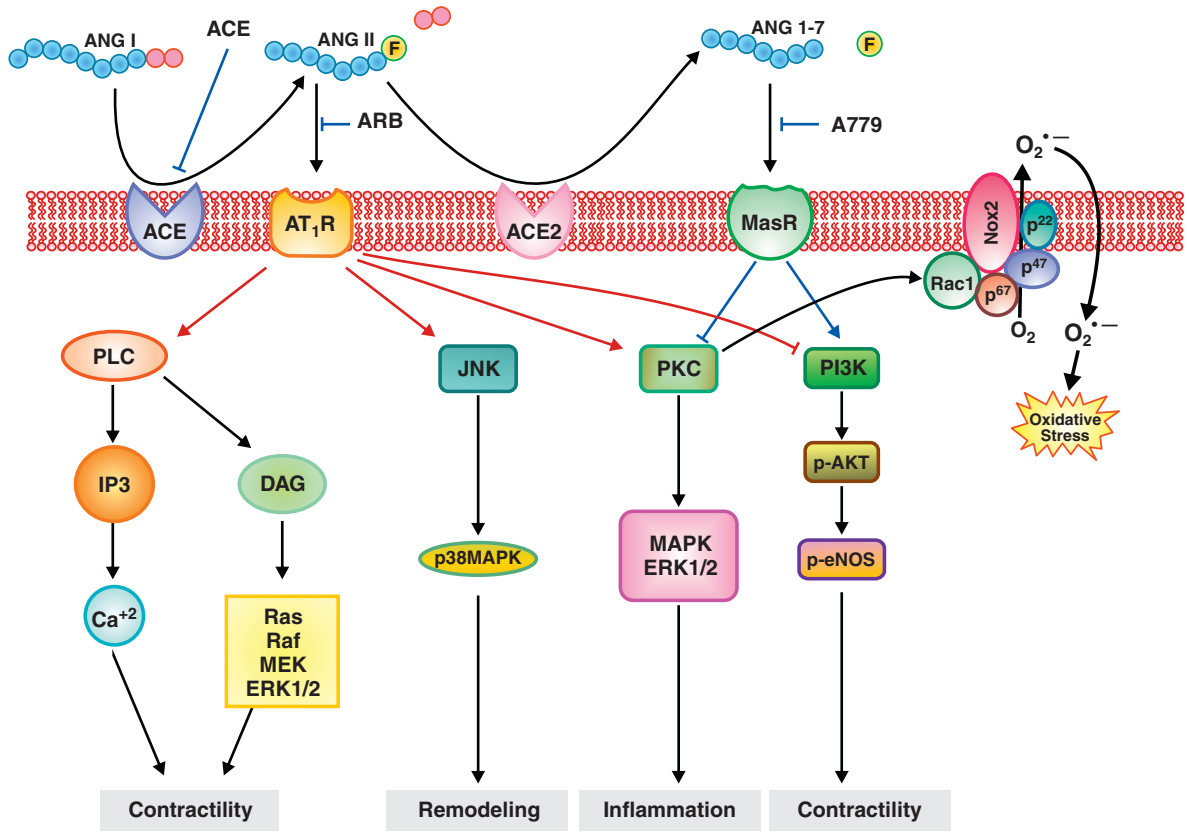

Figure 1. Diverse signaling pathways and cellular effects induced ACE/Ang II/AT_1R and opposing effects of ACE2/Ang (1–7)/MasR. ACE mediated generation of Ang II results in activation of various signaling pathways resulting in adverse cardiac remodeling and cardiac dysfunction. Activation of ACE2/Ang (1–7)/MasR axis counter-regulates Ang II/AT1R mediated effects and also stimulates PI3K-Akt-eNOS pathway mediated cardiac contractility. ACE: angiotensin converting enzyme; Ang 1–7: angiotensin (1–7); Ang II: angiotensin II; AT_1R: ang II type 1 receptor; ARB: AT_1 receptor blocker; DAG: diacyl glycerol; eNOS: endothelial nitric oxide synthase; ERK1/2: extracellular signal-regulated kinases 1/2; IP3: inositol triphosphate; MasR: Mas receptor; Nox2: NADPH oxidase 2; p38-MAPK: p38-mitrogen activated protein kinase; PKC: protein kinase C; PI3K: Phosphatidylinositide 3-kinase; PLC: phospholipase C.

relationship between myocardial ACE2 protein levels and disease progression [26].

We recently studied the role of ACE2 in the prevention of progression of diabetic cardiomyopathy using a type-1 Akita diabetic model [27]. We developed Akita/ACE2KO double mutant mice and studied them at 6 months of age. Loss of ACE2 resulted in systolic dysfunction with the background impaired diastolic function in the Akita hearts [27,28]. Akita/ACE2KO double mutant hearts showed increased NADPH oxidase activity resulting in increased ROS, protein kinase C and matrix metalloproteinases activity and enhanced cardiac extracellular matrix degradation leading to the progression of diabetic cardiomyopathy with systolic dysfunction. The role of ACE2 in ischemic cardiomyopathy has also been unveiled. Using the LAD-ligation model, we showed that ACE2KO mice had increased mortality with worsened heart function following myocardial infarction [29]. Myocardial levels of Ang II and Ang (1–7) were increased and decreased, respectively, resulting in greater inflammation, NADPH oxidase activation and degradation of the extracellular matrix. In consistent to our results, overexpression of ACE2 ameliorates left ventricular remodeling and dysfunction in a rat model of myocardial infarction [30].

Role of ACE2 in diastolic dysfunction

Diastolic heart failure and diastolic dysfunction, as defined by the American Heart Association to be 'heart failure with preserved ejection fraction (HFPEF),' is often associated with a normal or small heart size and diastolic filling abnormalities. Until recently, it was accepted that the prognosis for individuals with diastolic dysfunction and associated pulmonary edema was better than those with systolic dysfunction. Recent epidemiological studies of heart failure demonstrated that HFPEF accounts for approximately 50% of all heart failure with a similar mortality rate to patients with systolic heart failure [31,32]. We have studied the role of ACE2 in the clinically relevant animal model of Ang II infusion in mice which results in the increased plasma and myocardial Ang II levels. ACE2 negatively regulates the pathophysiological effects of a pressor and sub-pressor dose

of Ang II on myocardial structure and function [26]. Loss of ACE2 resulted in worsening of cardiac fibrosis and pathological hypertrophy resulting in worsened diastolic dysfunction in response to Ang II infusion. However, increased ACE2 action by the use of hrACE2 lowered plasma and myocardial Ang II levels and increased plasma Ang 1–7 levels *in vivo* providing definitive evidence for a key role of ACE2 in the metabolism of Ang II [26]. Pharmacological treatment with rhACE2 attenuated myocardial fibrosis, pathological hypertrophy and diastolic dysfunction seen in response to Ang II infusion. Cardiac fibroblasts express AT1 receptors and Mas receptors and Ang II is known to induce production of collagen and TGFβ1 [33,34] both of which were largely inhibited by rhACE2 [26]. Thus, rhACE2 treatment antagonized the activated RAS via switch in the biochemical milieu from ACE/Ang II/AT_1R arm to Ang (1–7)/MasR arm. Importantly, rhACE2 did not alter baseline plasma Ang II, Ang 1–7 or blood pressure in WT mice suggesting that substrate availability is a limiting factor in ACE2 enzymatic activity [35]. Human recombinant ACE2 treatment also inhibited the effects of Ang II on murine adult ventricular cardiomyocytes and cardiac fibroblasts via an Ang (1–7)/MasR dependent manner [26]. In addition, ACE2 lentiviral transfection in cultured fibroblasts show a decreased production of collagen in response to acute hypoxic exposure [36]. In consistent to our results, overexpression of ACE2 protects the heart against myocardial injuries induced by Ang II infusion in rats [37]. We proposed the ACE2 as an essential negative regulator of RAS dependent diastolic heart failure predominantly in the setting of increased Ang II levels.

Role of ACE2 in kidney disease

The importance of RAS to the development and progression of kidney disease is well established. While the short term, activation of RAS is vital for maintaining renal homeostasis, this occurs at the expense of glomerular hypertension. Along with other important RAS components, ACE2 is also present in the kidney as a member of local renal RAS. ACE2 is highly expressed in mouse kidneys, where ACE2 activity is higher in mouse kidney cortex than in mouse heart tissue [38]. The role of renal RAS and renal ACE2 has been well studied using various animal models [39–42]. Loss of ACE2 exacerbates Ang II induced renal oxidative stress, inflammation and fibrosis, where hrACE2 treatment prevented these pathological changes (Fig. 2) [41]. In consistent with our results, Fang *et al.* (2013), using a murine model of renal ischemia-reperfusion injury, have shown that loss of ACE2 exacerbates the renal inflammation, oxidative stress and apoptosis in response to acute kidney injury [43]. Diabetic nephropathy is one of the most common causes of end-stage renal failure. We found that the loss of ACE2 accelerates the diabetic kidney injury and hrACE2 reduces the progression of diabetic nephropathy [40,42]. Patients with kidney dysfunction are at higher risk of adverse cardiovascular outcomes and frequently develop left ventricular hypertrophy. Burchill *et al.* (2008) have studied the role of ACE2 in the kidney injury induced cardiac remodeling using a rat model of acute kidney injury. They found that acute kidney injury led to adverse cardiac remodeling with increase in cardiac ACE2 gene expression and activity, which was thought to be a compensatory response [43]. However, further studies are required to explain the role of any possible crosstalk between renal and cardiac ACE2 in the kidney dysfunction induced adverse cardiac remodeling and heart failure.

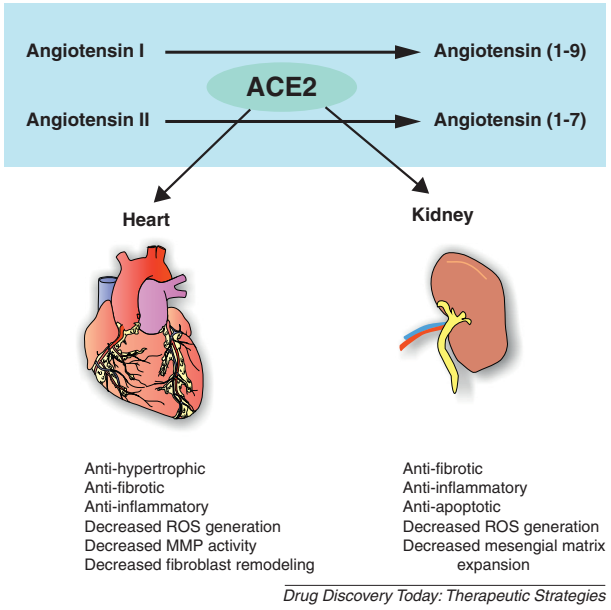

Figure 2. Summary of the effect of ACE2 on cardiac and renal systems. ACE2 is a type-I transmembrane enzyme which converts Ang I into Ang (1–9) and Ang II into Ang (1–7). ACE2 possesses anti-hypertrophic, anti-fibrotic, anti-inflammatory effects on the cardiac system attributed to decreased ROS production, decreased MMP activity and deceased fibroblast remodeling. ACE2 demonstrates anti-fibrotic, anti-apoptotic and anti-inflammatory effects along with decreased ROS production and decreased mesangial matrix expansion. MMP: matrix metalloproteinase; ROS: reactive oxygen species.

Use of human recombinant ACE2 as potential therapy for human heart failure

There are alternate pathways for converting Ang I to Ang II which do not require ACE [44]. ACE-independent Ang II formation was found to be particularly robust in the heart, and this activity was eventually assigned to a serine proteinase belonging to the chymase family [44,45]. Furthermore, the major cellular source of chymase is the mast cell [46,47]. Cardiac mast cells are also a source of renin thereby making a critical contribution to the local RAS in the heart [48]. Indeed, mast cells play a direct role in promoting left-ventricular dysfunction in a model of congestive heart failure [49]. A

clinical consequence of these pathways is seen in patients who take ACE inhibitors chronically, where incomplete suppression of Ang II levels in plasma is often observed [50–52]. The generation of tissue Ang II by non-ACE related enzymes such as chymase suggests that enhancing ACE2 action may indeed have a unique therapeutic role [26,47]. Ultimately, we need to better define the subpopulations of patients with heart failure in order to selectively target the appropriate subgroup where the ratio of Ang II/Ang 1–7 remains elevated.

In addition, blockade of angiotensin (Ang) II synthesis by angiotensin-converting enzyme (ACE) inhibitors or its actions by Ang II receptor blockers (ARBs) increases ACE2 expression in the heart [53–55]. Given the ability of ACE2 to protect against adverse myocardial remodeling, the increase in ACE2 mediated by ARBs and ACE inhibitions may in part explain their therapeutic benefits in patients with heart failure. In addition, upregulation of ACE2 activity in response to mineralocorticoid receptor blocker also occurs in patients with heart failure [56]. ACE2 is a type I membrane-anchored protein and has a catalytically active ectodomain that undergoes shedding due to the proteolytic action of tumor necrosis factor α-converting enzyme (TACE; Fig. 3) [57,58]. Hyperactivity of TACE-mediated cleavage might represent a mechanism by which localized RAS dysregulation occurs, which results in the neurohumoral imbalance that is typical of heart failure [59]. Inhibition of the cleavage of ACE2 from the surface of cardiac cells leading to retention of enzymatic activity, reduced Ang II and increased Ang 1–7 within the cardiac microenvironment may have therapeutic benefits. Animal model comparisons of cardiac-specific versus whole-body *Ace2* genetic knockouts, complemented by pharmacological studies comparing supplemental hrACE2 to gene-delivered *Ace2* will be necessary to support this hypothesis. Indeed, future development of ACE2 as a therapeutic agent will be aided by basic research of this nature. Strategies to retain ACE2 in the heart would need to account for the fact that ACE2 is cleaved at more than a single site and that multiple enzymes appear to serve as sheddases in this process. The central role of ADAM17, however, suggests that agents that block its activity could be effective ways of enhancing ACE2 activity in the heart [57,58]. Kinetic evidence lends support to the idea of maintaining cardiac ACE2, as Ang (1–7) is effectively cleaved to inactive Ang (1–5) by ACE [60]. Therefore, paracrine signaling, achieved by tethering ACE2/Ang (1–7)/Mas at the heart might be more potent than endocrine signaling caused by systemic Ang (1–7) production. The resolution of gene delivery of *ACE2* or rhACE2 might simply be a problem of dosing: since solubilized ACE2 has the entire intact catalytic site and shares substrates with tissue-bound ACE2, rhACE2 would need to be dosed such that Ang (1–7) reaching the heart achieves similar benefit as if it were produced there [61].

Human recombinant ACE2 was administered intravenously to healthy human subjects in a randomized, double-blinded, placebo-controlled study [62]. 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 [63]. Single rhACE2 doses of 100–1200 μg/kg caused a dose-dependent increase of systemic exposure with biphasic elimination and a dose-independent terminal half-life of 10 hours. In all single-dose cohorts, Ang II decreased within 30 min post-infusion, Ang1–7 either increased (100 and 200 μg/kg doses), decreased, or remained unchanged (400–1200 μg/kg doses), whereas angiotensin 1–5 (Ang1–5) transiently increased for all doses investigated [62]. With the exception of the lowest rhACE2 dose, the decrease in Ang II levels lasted for at least 24 hours. Administration of rhACE2 was well tolerated by healthy human subjects with a dose-independent terminal elimination half-life in the range of 10 hours [62]. Despite marked changes in angiotensin system peptide concentrations, cardiovascular effects were absent, suggesting the presence of effective compensatory mechanisms in healthy volunteers.

Analysis of ACE2 activity and its role as a biomarker in patients with heart failure

There are multiple lines of evidence examining the RAS that collectively point to expression of ACE2 in many tissues and organ systems, including brain, retina, adipose tissue, gastrointestinal tract, liver, heart, kidney and blood vessels [64–68]. However, the tissues of principal interest with respect to the potential of ACE2 as a biomarker in heart failure are the heart, kidneys, and vascular endothelia. Analysis of ACE2 activity may be carried out in tissue or plasma samples; analysis in plasma samples is less invasive and can be much more widely applied, while analysis in tissues, though invasive, gives specific evidence of changes in a single system under study, such as the myocardium, which is important for analyzing the relative changes in RAS activity within pathologies involving multiple organ systems (Fig. 3). Nonetheless, ACE2 has been more widely studied as a plasma biomarker. Previous work shows that ACE2 can be reliably detected in human plasma, and in an analysis of a cohort of individuals suspected of having HF, plasma ACE2 activity increased with an increasing proportion of confirmed HF diagnoses, and also increased in response to worsening disease status, as indicated by New York Heart Association functional class [69]. Further investigation determined that plasma ACE2 activity was significantly associated with left-ventricular ejection fraction and BNP among other parameters measuring heart function in a cohort of patients with HF with reduced left-ventricular ejection fraction (HFREF) [70]. Recent evidence from patients admitted to hospital with STEMI indicated that plasma ACE2 negatively correlated to ejection fraction and positively correlated to infarct size at both initial presentation

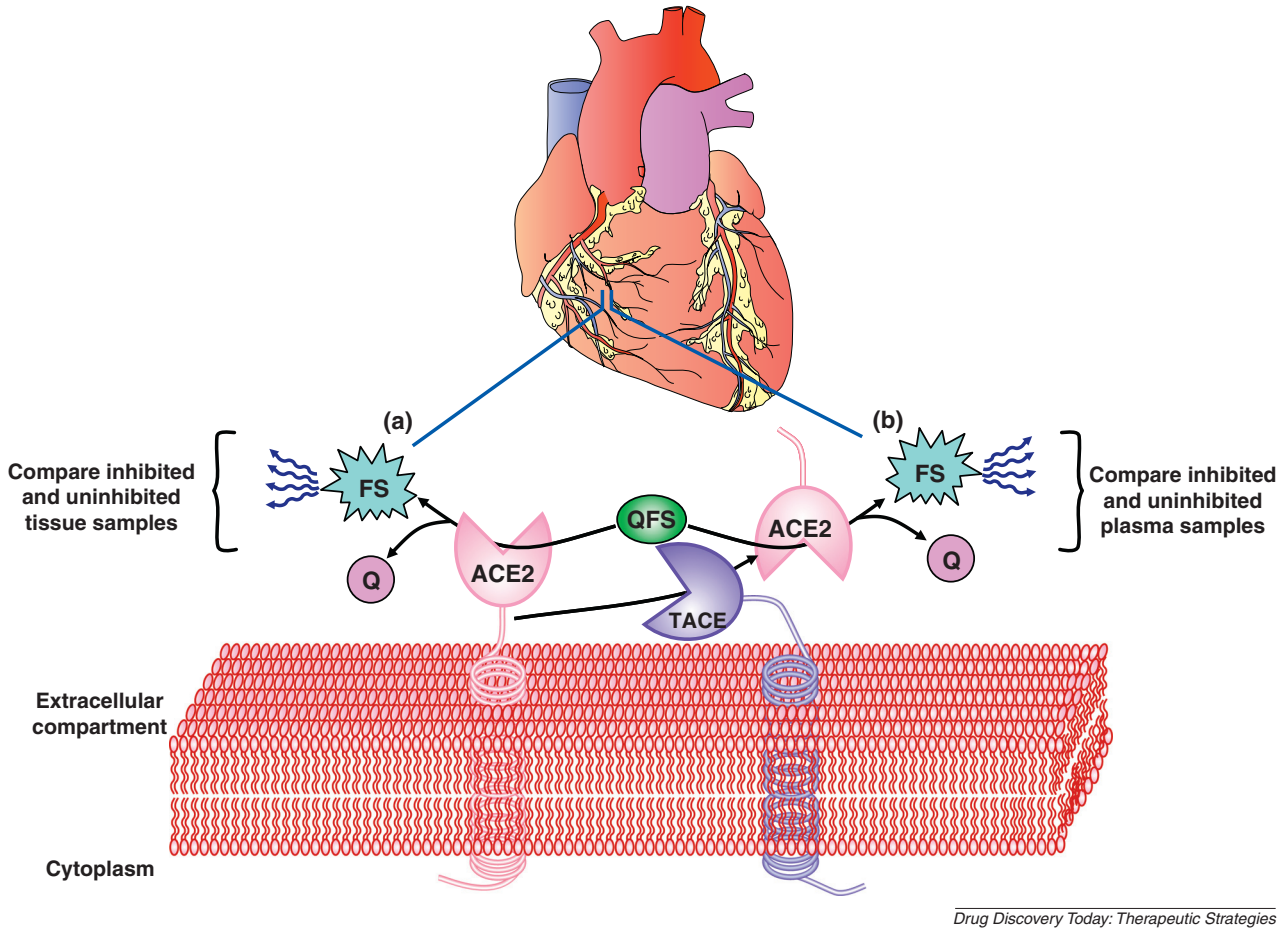

Figure 3. The principle of ACE2 activity assays. **(a)** ACE2, a membrane-bound carboxypeptidase, metabolizes quenched fluorescent substrate (QFS), to produce a fluorescent moiety which is then detected. Activity of the tissue sample is then determined using the difference in fluorescence between inhibited and uninhibited samples. **(b)** TACE/ADAM17, another membrane-bound protease, cleaves off the enzymatically active N-terminal domain of ACE2. Circulating ACE2 can be detected in plasma samples using a similar fluorescence protocol. TACE/ADAM17-mediated cleavage of ACE2 is also a mechanism by which RAS dysregulation is thought to occur in cardiac pathologies.

and follow-up [71]. The results from analyses of ACE2 activity in HFREF show the strong potential for ACE2 as a prognostic biomarker in HF; however, these data omit those patients with HFPEF, who make up a significant portion of the total heart failure population [31,32]. To date, no studies have specifically addressed plasma ACE2 activity in an HFPEF cohort; therefore, the lack of ACE2 activity analyses focused on HFPEF identifies a critical need moving forward with the analysis of ACE2. The heterogeneity between HFREF and HFPEF is well documented, so it is impossible to suggest that generalizations regarding HFREF can be wholly applied to HFPEF [31,32]. Critical to the development of ACE2 activity as a biomarker in heart failure will be evaluating the interplay between tissue activity and plasma activity – to date, studies have been concerned with one or the other. The elevation of plasma ACE2 activity in heart failure might reflect either increased shedding or increased expression, or perhaps a combination of both. There is evidence from RT-PCR analyses that expression of the *ACE2* gene is upregulated in the human failing heart [23], but this not disqualify increased tissue shedding as another mechanism by which plasma ACE2 activity is increased in cases of heart failure. However, complicating the issue of resolving the interplay between plasma and tissue ACE2 is evidence that an endogenous ACE2 inhibitor exists [72]. Despite these hurdles, analyses of ACE2 in heart failure have thus far been encouraging, and there is reason to continue pursuing ACE2 as a biomarker in heart failure.

Conclusions

Angiotensin-converting enzyme 2 negatively regulates the RAS. ACE2 cleaves Ang II into the biologically active, Ang 1–7. ACE2 is highly expressed in the heart and the vasculature including the endothelium. Loss of ACE2 enhances the susceptibility to cardiovascular disease including heart failure while enhancing ACE2 action prevents adverse pathological remodeling and lessens the progression to heart failure. Thus, drugs targeting ACE2 such as use of hrACE2 represent

potential candidates to prevent and treat diastolic and systolic heart failure. Indeed, rhACE2 has been shown to lower plasma Ang II levels in healthy human volunteers. Elevated Ang II levels despite ACE inhibition can occur via the chymase system which can be targeted by rhACE2. As we move forward, any novel therapy aimed at enhancing ACE2 action will have to be tested against ACE inhibition, angiotensin receptor blocker, beta-blockers all of which have been shown to reduce the morbidity and mortality in patients with heart failure.

Conflict of interest

None

Acknowledgements

Gavin Y. Oudit is a Clinician-Investigator of the Alberta Innovates-Health Solutions and the Distinguish Clinician Scientist of the Heart and Stroke Foundation of Canada and Canadian Institutes of Health Research. Vaibhav B. Patel is supported by AI-HS Post-Doctoral Fellowship and Heart and Stroke Foundation of Canada Fellowships. Our research activities have been supported by operating grants from the Heart and Stroke Foundation of Canada and Canadian Institutes of Health Research.

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