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ACE2: Angiotensin II/Angiotensin-(1–7) Balance in Cardiac and Renal Injury

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Abstract Our current recognition of the renin-angiotensin system is more convoluted than originally thought due to the discovery of multiple novel enzymes, peptides, and receptors inherent in this interactive biochemical cascade. Over the last decade, angiotensin-converting enzyme 2 (ACE2) has emerged as a key player in the pathophysiology of hypertension and cardiovascular and renal disease due to its pivotal role in metabolizing vasoconstrictive/hypertrophic/proliferative angiotensin II into favorable angiotensin-(1–7). This review addresses the considerable advancement in research on the role of tissue ACE2 in the development and progression of hypertension and cardiac and renal injury. We summarize the results from recent clinical and experimental studies suggesting that serum or urine soluble ACE2 may serve as a novel biomarker or independent risk factor relevant for diagnosis and prognosis of cardiorenal disease. We also review recent proceedings on novel therapeutic approaches to enhance ACE2/angiotensin-(1–7) axis.

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Introduction

An ever-emerging body of experimental and clinical evidence continues to support a key role of the renin-angiotensin system (RAS) in the pathogenesis of hypertension. In the last decade, our understanding of the convoluted RAS has expanded onto the existence of novel angiotensins that counteract the hypertensive, growth-promoting, and proliferative effects of angiotensin II (Ang II). Indeed, angiotensin-(1–7) [Ang-(1–7)], its forming enzyme angiotensin-converting enzyme 2 (ACE2), and receptor Mas have been a topic of interest not only in hypertension research but also across different research areas, reflecting pleiotropic effects of RAS effector hormones. This review addresses the considerable advancement of knowledge on these novel components of the RAS, with focus on ACE2. The significance of this progress is made apparent from a search of papers in PubMed over the last three years with terms such as ACE2, heart, and kidneys. These keywords yielded over 200 publications.

The Conventional vs. Alternate RAS

The conventional RAS has been viewed as a classical hormonal system comprising the enzymatic cleavage of the decapeptide angiotensin I (Ang I) in the circulation by renal renin from liver-derived angiotensinogen. Further cleavage of two amino acids from the C-terminal part of Ang I by angiotensin-converting enzyme (ACE), primarily in the pulmonary circulation, leads to formation of Ang II, which contributes to the regulation of blood pressure by influencing

vascular smooth muscle cells and sodium and volume homeostasis as well as aldosterone secretion. The Ang II effects are mediated through its two known plasma membrane receptors, angiotensin type 1 (AT1) and AT2 receptors. There is some controversy about the outcome of AT2 activation, but a majority of reports points toward opposing actions of AT2 on vascular tone and sodium homeostasis.

As opposed to this classical endocrine system, where the action of the hormone takes place quite remotely from its origin, the concept of the local-tissue RAS with paracrine, autocrine, and intracrine actions has become increasingly appreciated in the last two decades, underlining the role of the RAS in regulating cell growth and proliferation, inflammation, and cytokine production. Indeed, a growing body of evidence testifies that each and every component of the RAS is found throughout diverse tissues and organs, including the heart, vasculature, kidneys, brain, lung, and reproductive tissues. Importantly, recent studies identifying new enzymes (ACE2) or new substrates for known enzymes (chymase, ACE), peptides [Ang-(1–12), Ang-(1–9), Ang-(1–7)], and receptors (renin/prorenin receptor, Mas receptor) have brought novel insight into the role of the RAS in pathophysiology of hypertension and related cardiovascular and renal disease. In healthy and diseased human heart, for example, we recently showed a role for chymase in the formation of Ang II from Ang-(1–12), a new precursor for downstream angiotensin peptides [1•, 2, 3]. In this review, we will provide an update on ACE2 counteracting the majority of Ang II cardiovascular and renal effects, as well as its usefulness as a novel biomarker and therapeutic target for cardiovascular and renal disease.

ACE2/Ang-(1–7)/mas axis

The heptapeptide Ang-(1–7) [Asp^1-Arg^2-Val^3-Tyr^4-Ile^5-His^6-Pro^7-] is a truncated form of Ang II, lacking phenylalanine in the eighth position. Its functional role in counterbalancing Ang II actions was recognized long before the discovery of its forming enzyme ACE2 and related receptor Mas. As previously reviewed [4, 5], Ang-(1–7) induces systemic and regional vasodilation, diuresis and natriuresis, and exerts antiproliferative and antigrowth effects in vascular smooth muscle cells, cardiac myocytes, and fibroblasts as well as glomerular and proximal tubular cells. Cardiorenal protective effects of Ang-(1–7) are mediated by the Mas receptor through different signaling pathways, including several autocoids, mitogen-activated protein kinase (MAPK), AKT, NADPH oxidase, transforming growth factor (TGF)-β1, epidermal growth factor (EGF) receptor, and NF-kapaB activity.

ACE2 was discovered in 2000 as a homologue enzyme to the better-known ACE, sharing many features of the enzymes belonging to a family of zinc metalloproteinases. Similar to ACE, ACE2 is a plasma membrane-bound ectoenzyme, although soluble forms in plasma and urine are also found [6, 7•]. Shedding of ACE2 has frequently been associated with the activity of tumor necrosis factor alpha-converting enzyme (TACE) [8]. In contrast to ACE, which has two active-site domains and acts as dicarboxipeptidase, ACE2 expresses only one catalytic site and acts as a monocarboxypeptidase, removing one amino acid from the C-terminus of its substrates. ACE2 metabolizes Ang I and Ang II into Ang-(1–9) and Ang-(1–7), respectively, with higher preference for Ang II degradation [9]. Other known ACE2 substrates belong to the apelin family (apelin 13, apelin 17, apelin 36) which, in addition to Ang-(1–7), exert important protective cardiovascular actions [10]. ACE2 is also insensitive to the actions of known ACE inhibitors [11, 12].

ACE2 and Cardiac Disease

ACE2 is widely distributed, and in the heart, its presence has been documented in the coronary vessels as well as in cardiac myocytes and fibroblasts. The first study using ACE2 knockout (ACE2 KO) mice testified to the importance of this intracardiac enzyme in regulation of cardiac structure and function. ACE2 gene deletion resulted in major defect in cardiac contractility associated with increased plasma and heart Ang II [13]. Subsequent studies from our laboratory showed ACE2-dependent generation of Ang-(1–7) from Ang II in isolated hearts from mRen2 transgenic rats [14]. In the same animal model, we further showed that chronic ACE2 inhibition with MLN-4760 for 28 days worsened cardiac remodeling that was associated with increased cardiac Ang II levels [15]. Genetic ablation or pharmacological inhibition of ACE2 has been also associated with exacerbation of left ventricular remodeling and dysfunction in response to myocardial infarction [16, 17], diabetes [18•], and aging [19]. In agreement with these studies are the recent reports on significant reduction of ACE2 protein in response to pressure overload [20•] and the further worsening of cardiac remodeling and development of systolic dysfunction, and consequent heart failure, due to biomechanical stress imposed on the background of ACE2 gene deletion [21, 22]. Cardiac remodeling and dysfunction under these conditions were linked to protein kinase C-mediated activation of $p47^{phox}$ NADPH oxidase subunit, resulting in augmented oxidative stress, and extracellular signal-regulating protein kinases (ERK)1/2 activation, as well as increased matrix metalloproteinase 2 and 9 activities. Moreover, a critical role of increased action of Ang II and/or loss of Ang-(1–7) effects has been proven in a subsequent report, where blockade of AT1 receptors by irbesartan or infusion of Ang-(1–7) exerted a comparable level of cardioprotection in pressure-overloaded ACE2 null mice [23•]. Beneficial signaling pathways were confirmed in cultured cardiac myocytes and fibroblast isolated from pressure-

overload ACE2 KO mice. Similarly, irbesartan reduced systolic dysfunction in diabetic ACE2 KO mice, reducing the enhanced activation of NADPH oxidase and metalloproteinases [18•]. Thus blockade of Ang II action or Ang-(1–7) supplementation may be an effective treatment for heart failure associated with ACE2 downregulation.

On the other side, increased ACE2 mRNA, protein, and activity were reported in failing human hearts [24–27] and several experimental models of myocardial infarction [16, 25]. Moreover, increased ACE2 activity in the plasma of heart failure patients correlates with unfavorable clinical outcomes [6, 28•]. It seems that increased ACE2 levels and Ang-(1–7) formation are compensatory mechanisms to impede the progression of heart failure, keeping in mind that ACE2 overexpression provided cardioprotection in rats subjected to myocardial infarction [29]. Importantly, our laboratory documented the beneficial upregulation of ACE2 mRNA in infarcted rat hearts after 28 days of treatment with AT1 receptor antagonists [30]. In cardiac remodeling following prolonged nitric oxide inhibition [31] or heart failure due to experimental myocarditis [32], cardioprotective effects of AT1 receptor blockade were also mediated through ACE2/Ang-(1–7) axis. These data are in agreement with several in vivo and in vitro reports documenting negative ACE2 regulation by Ang II and endothelin through activation of MAPK pathways [33–37]. Moreover, Ang-(1–7)-mediated MAPK phosphatase activation counteracted inhibitory effects of Ang II on ACE2 in cardiomyocytes and vascular smooth muscle cells [36, 38], while lentivirus-mediated overexpression of Ang-(1–7) increased ACE2 gene expression in cardiac tissue of rats subjected to coronary artery ligation [39]. In agreement with the data, Ang-(1–7) increased ACE2 expression in neonatal cardiac myocytes under hypoxic conditions [39]. However, a recent report that both Ang II and Ang-(1–7) positively regulated ACE2 [40] in human cardiac fibroblasts warrants further studies in addressing ACE2 regulation and related signaling pathways.

An increasing body of evidence suggests that ACE2 gene polymorphisms are associated with left ventricular hypertrophy [41–44], coronary heart disease and myocardial infarction [45], cardiac remodeling, and urinary protein level in men with type 2 diabetes and coronary heart disease [46], as well as with hypertension [42, 47, 48], hypertension in women [49], and antihypertensive response to ACE inhibitors in women [50]. On the other hand, some reports found no association between ACE2 and hypertension [51, 52] and ACE2 and risk for sudden cardiac death in women or men [53], underscoring the necessity for further evaluation of the impact of ACE2 gene variants on cardiovascular disease in the human population.

Renoprotective Role of ACE2

In human and rodent kidney, ACE2 was found in glomerular podocytes and mesangial cells and along different tubular segments of the nephron. Several studies pointed to the lack of ACE2 expression in endothelium of glomerular capillaries and small renal arterioles [54–56], while ACE2 is highly expressed in vascular smooth muscle cells and endothelium of larger interlobular arteries [54]. Most of the studies stressed a much higher expression in tubules as compared to glomerulus [54, 56–58] and ACE2 expression in mouse kidney was found to be 20-fold higher than in mouse heart tissue [59].

Such a high and wide distribution throughout the nephron unit testifies to the importance of the ACE2 contribution to the maintenance of renal hemodynamics and tubulo-glomerular function. Indeed, genetic, pharmacological, and functional loss of ACE2 resulted in increased albuminuria and progression to glomerulosclerosis. ACE2 genetic ablation resulted in subtle fibrillar collagen accumulation in glomerular mesangium seen only under electron microscope in early age, while it led to severe glomerulosclerosis in aging mice [60]. Moreover, when ACE2 KO mice were crossed with Akita mice (a model of type 1 diabetes), more advanced glomerular damage was induced independently of blood pressure and glucose levels [61]. Similarly, ACE2 deletion in mice subjected to unilateral ureteral obstruction increased Ang II and decreased Ang-(1–7) in obstructed kidney, leading to exaggerated renal inflammation and fibrosis associated with enhanced TGF-β/Smad2/3 and NF-kappaB signaling pathways [62]. Kidney Ang II was consistently many times higher, and hypertension, renal oxidative stress, and inflammation were more severe, in ACE2 KO than wild-type mice following Ang II infusion, confirming the critical role of ACE2 in the regulation of kidney Ang II metabolism, blood pressure, and tissue damage [63, 64]. In agreement, pharmacological inhibition of ACE2 by MLN-4760 worsened albuminuria in two mouse model of diabetes, db/db mice [56] and diabetes induced by streptozotocin (STZ) [65]. Although renal Ang II content and intrarenal Ang II signaling were not consistently evaluated in these studies, specific AT1 receptor antagonists ameliorated or prevented renal damage in some of the studies [56, 60, 61], demonstrating that development of renal pathology was, indeed, Ang II-mediated.

In many models of genetic or induced hypertension, kidney ACE2 was decreased in adult animals [13, 66, 67]. Importantly, in the kidneys of spontaneously hypertensive rats (SHR), an excellent model of essential hypertension in humans, the developmental pattern of ACE2 expression was altered before the onset of hypertension, suggesting a causative role of altered ACE2 expression in the development of hypertension [68]. Increased salt intake and related kidney damage were associated with reduced cortical expression of ACE2 in obese Zucker rats [69] and increased ACE/ACE2 mRNA and protein ratio in glomeruli of uninephrectomized WKY [70], respectively. The similar ratio between ACE and ACE2 was also found in renal biopsies from patients with hypertension when compared to subjects with normal blood pressure [71], as well as in patients

with IgA nephropathy [72] and diabetes [57, 73]. No differences in ACE2 expression between patients with focal segmental glomerulosclerosis and control cohort has been reported [73]; these data suggest that decrease in ACE2 is not a general response to kidney injury. In contrast to clinical studies on diabetic patients, there are some discrepancies with regard to the expression and activity of ACE2 in experimental diabetes [56, 58, 59]. This may have stemmed from differences in animal models and nephron segment studied as well as the degree of the disease. Nevertheless, reduced ACE2 expression and activity in glomeruli was consistently found in experimental diabetes as well [56, 58]. Importantly, podocyte-specific overexpression of human ACE2 transiently attenuated the development of STZ-induced diabetic nephropathy [74•], providing support for the crucial role of glomerular ACE2 in the onset of diabetes-related kidney disease. Furthermore, in contrast to the association between ACE2 gene variants and hypertension or cardiac disease, no association was found between ACE2 gene polymorphisms and diabetic nephropathy or complications such as increased blood pressure or hemoglobin A1C [75, 76].

Reperfusion of ischemic kidney led to decrease in mRNA for ACE2 and consecutive changes in renal Ang II and Ang-(1–7), as did subtotal nephrectomy [25, 77, 78]. There were some discrepancies as to whether Ang-(1–7) supplementation was beneficial under the condition of nephron loss [79], but it was demonstrated that both Ang-(1–7) and losartan normalized cortical ACE2 expression [77]. Despite normalization of plasma Ang II by Ang-(1–7), there were no changes in blood pressure, albuminuria, and glomerular filtration rate consistent with increased mesangial area in glomeruli of rats treated with the heptapeptide [77]. Indeed, growth-stimulating effects of Ang-(1–7) associated with extracellular matrix protein production were demonstrated in human mesangial cells [80]. On the other hand, acute renal injury due to limb ischemia-reperfusion in mice is exacerbated with ACE2 deletion and rescued with its overexpression, which correlates with adequate changes in circulating but not tissue Ang II and Ang-(1–7) [81]. Nevertheless, these studies add to the evidence that targeting ACE2 in Ang II degradation and/or Ang-(1–7) formation might be a valuable therapeutic approach for renal disease of different etiologies.

Our laboratory was among the first to show that ACE inhibitors or AT1 receptor antagonist given for two weeks enhanced renal ACE2 activity that was associated with increased Ang-(1–7) in plasma and urine of normotensive animals [33]. The concomitant activation of unopposed AT2 receptors contributing to the ACE2 upregulation by AT1 receptor blockade cannot be ruled out. Indeed, a study published just a month ago revealed ACE2 and Ang-(1–7) upregulation in response to treatment with an AT2 agonist in obese Zucker rats that was associated with reduction of blood pressure and increased urinary sodium excretion [82•]. Other studies in rodents [78] and humans with non-diabetic kidney disease [73] has suggested that upregulation of ACE2 may have abated the progression of renal disease. These studies are in accord with in vitro reports that Ang II, in AT1 fashion, downregulated ACE2 in human tubular cells via MAPK (ERK1/2 and p38) pathway [83]. However, our most recent study, in which we employed six-week treatment with olmesartan, showed that a marked reduction of blood pressure was associated with increased renal ACE2 protein, but not activity, in hypertensive mRen2.Lewis rats [84]. Posttranscriptional changes were suggested in studies in which a discrepancy was also found between ACE2 mRNA, protein, and/or activity in kidneys of diabetic animals [59, 85]. Together, these studies strongly suggest that a pathophysiological role of ACE2 should be assessed through comprehensive analysis, including not only its expression on the level of mRNA and protein but also enzymatic activity.

Circulating and Urinary ACE2: Novel Biomarker for Cardiac and Renal Disease?

As it was previously mentioned, a cleavage of the catalytically active ectodomain of ACE2 results in a smaller protein fragment found in plasma, serum, and urine of humans and experimental animals. The very low ACE2 activities were detected in human plasma of healthy individuals and were frequently related to the presence of an endogenous inhibitor [86, 87]. In contrast, increased serum ACE2 activities were measured in patients with acute and chronic heart failure [6, 28, 88–90] and were correlated positively with plasma B-type natriuretic peptide (BNP), a potent predicting factor of mortality and morbidity in heart failure [6, 28, 88]. Higher ACE2 activity was associated with more severe disease and lower ejection fraction, regardless of the presence or absence of ischemic disease; in another study, however, increased ACE2 activity may have reflected coronary heart disease associated with diabetes [91]. In patients with chronic systolic heart failure, plasma ACE2 activity was predictive of unfavorable clinical outcome independently of ejection fraction and BNP. Moreover, it was suggested that measurements of both ACE2 activity and BNP may be of greater value in predicting the occurrence of adverse cardiac events [6, 28].

With regard to renal disease, augmented serum ACE2 activity was associated with microalbuminuria in patients with type 1 diabetes [91]. In addition, urinary ACE2 correlated positively with the degree of proteinuria [92] and albuminuria [93] in patients with type 2 diabetes, translating findings of increased ACE2 protein and activity in urine [7] and serum [94] of diabetic experimental models. While there are some differences in the expression of renal ACE2 between human and experimental diabetic nephropathy in the current literature, a study by Wysocki et al. clearly suggests that urinary ACE2

reflected renal rather than systemic source [7]. Few studies reported gender differences with respect to circulating ACE2; ACE2 was higher in males vs. females in healthy subjects and in patients with renal disease [87, 91, 95], mimicking gender disparity in the development of cardiovascular disease. Together, these studies suggest that measurement of soluble ACE2 may be a helpful diagnostic and prognostic marker for patients with cardiovascular and/or renal disease. While it is still unknown whether increased soluble ACE2 originated from increased tissue synthesis or augmented tissue shedding, it may reflect a compensatory, albeit still insufficient, response to adverse stimuli. Further increase in serum ACE2 after therapeutic intervention in patients with acute decompensated heart failure was associated with favorable clinical outcomes [90], providing additional support for therapeutic approaches to increase ACE2 activity in various diseases.

Novel Therapeutic Advances to Enhance ACE2/Ang-(1–7) axis

An increasing body of evidence suggests that novel therapeutic approaches to augment ACE2, and consequently decrease Ang II while increasing Ang-(1–7) actions, may be particularly beneficial in multiple disease states associated with elevated Ang II/Ang-(1–7) ratio. Our laboratory was among the first to show that classical ACE inhibitors and AT1 receptor antagonists also augmented ACE2 activity in heart and kidneys of normotensive and hypertensive rats as well as in the heart of rats with myocardial infarction [30, 33, 34, 37]. As exercise is well accepted as one of the most powerful lifestyle interventions in the treatment of hypertension and cardiovascular disease, it is important to note that cardiac ACE decreased and ACE2 increased following exercise in both lean and obese rat strains [96]. Thus, the reciprocial changes in ACE/ACE2 were recognized as the underlying cardioprotective molecular mechanisms for development of non-pathological left ventricular hypertrophy in response to aerobic exercise, and the regulatory role of specific microRNAs was suggested [97•].

In most recent studies, recombinant human ACE2 (rhACE2) concealed Ang II induced cardiac remodeling and dysfunction and related pathological signaling events in ACE2-deficient mice, decreasing plasma and tissue Ang II and increasing plasma Ang-(1–7) [20•]. It also attenuated the development of dilated cardiomyopathy in pressure-overloaded wild-type mice. Moreover, Ang-(1–7) mediated the effects of rhACE2 in suppressing Ang II-induced oxidative stress, expression of profibrotic genes, and ERK1/2 signaling in cultured cardiomyocytes and fibroblasts [20•]. Lower levels of Ang II and increased Ang-(1–7) were consistently associated with slower progression of diabetic nephropathy due to supplementation with rhACE2 in a murine experimental model of type 1 diabetes [98]. In SHR, rhACE2 attenuated hypertension and cardiac, kidney, and vascular oxidative stress [99], while both human [100] and mouse rACE2 [101] attenuated Ang II-induced hypertension by decreasing plasma Ang II.

In keeping with the above-referenced findings, overexpression of ACE2 by adeno- or lentivirus transfection exerted cardiac protection in Ang II-infused rats [102], rats with myocardial infarction [29] and diabetic cardiomyopathy [103], as well as renoprotective action in diabetic nephropathy [104]. Moreover, podocyte-specific overexpression of hACE2 transiently attenuated the development of diabetic nephropathy [74•], reflected in early protection from albuminuria, partial preservation of podocyte number and specific podocyte proteins nephrin and synaptopodin, as well as decreased profibrotic TGF-β1. Similarly, novel compounds with ACE2 activation ability reduced blood pressure in SHR [105] and improved cardiac function in diabetic rats [106], although further studies are necessary to confirm that the effects are, indeed, related to the relevant changes in Ang peptides.

Relative failure of classical RAS blockade in preventing development of end-stage heart and kidney disease could be explained, at least in part, by incomplete suppression of Ang II in response to the therapy with ACE inhibitors or AT1 receptor antagonists. Moreover, there are some conflicting reports as to the ability of the RAS blockade to upregulate ACE2 to facilitate Ang II metabolism [84, 107]. Therefore, more complete Ang II inhibition may be achieved by combining classical RAS blockade with novel approaches to enhance ACE2 activity, facilitating Ang II degradation. We recently reviewed alternative non-ACE-related pathways for Ang II production in human and experimental heart disease, including the activity of chymase on Ang-(1–12), a new precursor for downstream angiotensin peptides [1•, 2, 3]. Other studies have also reported the importance of chymase derived from mast cells in development of left ventricular dysfunction [108]. In this context, in addition to classical blockade of Ang II synthesis and action by ACE inhibitors or AT1 receptor blockers, therapeutic approaches to augment ACE2 and consequently decrease Ang II while increasing Ang-(1–7) actions may be particularly beneficial under conditions when these alternative Ang II synthetic pathways are overactive.

Conclusion

Experimental and clinical studies continue to provide novel evidence on the crucial role of ACE2/Ang-(1–7) in counterbalancing vasoconstrictor/hypertrophic/proliferative effects of Ang II determining the onset and progression of hypertension and cardiac and renal damage. Additional studies are needed to advance initial progress on the pharmacological and genetic therapeutic approaches to enhance ACE2 activity,

aiming to decrease Ang II while increasing Ang-(1–7) actions. This may be of particular interest when complete suppression of Ang II in response to therapy with ACE1 or AT1 receptor antagonist is not achieved or when alternative Ang II synthetic pathways are overactive. Further research is needed to confirm whether serum or urine soluble ACE2 may serve as a novel biomarker or independent risk factor relevant to diagnosis and prognosis of cardiorenal disease.

Compliance with Ethics Guidelines

Conflict of Interest Jasmina Varagic, Sarfaraz Ahmad, Sayaka Nagata, and Carlos M. Ferrario declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent With regard to the authors' research cited in this paper, all procedures were followed in accordance with the ethical standards of the responsible committee on human experimentation and with the Helsinki Declaration of 1975, as revised in 2000 and 2008, and all institutional and national guidelines for the care and use of laboratory animals were followed.

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