Pharmacologic Modulation of ACE2 Expression

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Angiotensin-converting enzyme 2 (ACE2) is an enzymatically active homologue of angiotensin-converting enzyme that degrades angiotensin I, angiotensin II, and other peptides. Recent studies have shown that under pathologic conditions, ACE2 expression in the kidney is altered. In this review, we briefly summarize recent studies dealing with pharmacologic interventions that modulate ACE2 expression. ACE2 amplification may have a potential therapeutic role for kidney disease and hypertension.

Introduction

Angiotensin-converting enzyme 2 (ACE2) is a carboxypeptidase that cleaves the peptides angiotensin (Ang) I and II, apelin, and neurotensin [1]. The *ACE2* gene is mapped in the human X chromosome (Xp22) and contains 18 exons, somewhat similar to ACE in exon size and organization [2]. The human ACE2 protein is a zinc-containing metallopeptidase with 806 amino acids and shares 42% sequence identity and 61% sequence similarity with ACE [2,3]. Within the renin-angiotensin system (RAS), ACE2 promotes the degradation of the vasoconstrictor and proliferative peptide Ang II to the vasodilatory and antiproliferative Ang-(1-7) [4].

ACE2 expression was initially found in the testis, kidney, and heart [2,3]. Later studies showed widespread ACE2 distribution in the lung, liver, small intestine, brain, and placenta [5,6•,7–9,10•]. The distribution of ACE2 in the kidney is specifically found in the apical membranes of the proximal tubules and in the glomerular epithelial cells (podocytes) [11,12]. ACE2 alterations have been described in experimental models of hypertension and diabetic kidney disease [13•–15•,16••]. ACE2 was found to be decreased in the setting of hypertension. Crackower et al. [4] showed that ACE2 was reduced in kidneys from three separate hypertensive rat strains: salt-sensitive Sabra hypertensive rats (SBH/y), spontaneous hypertensive rats (SHR), and stroke-prone spontaneously hypertensive rats (SHR-SP). Kidney ACE2 gene and protein expression were decreased in adult SHR as compared with Wistar-Kyoto (WKY) rats [17].

Our laboratory showed that in the db/db murine model of experimental diabetes, ACE2 glomerular expression is decreased by immunohistochemistry [16••]. In agreement with this finding, Leehey et al. [18] also found decreased glomerular ACE2 protein levels and activity in streptozotocin-induced diabetic rats. The tubular expression of ACE2 is altered, and the glomerular expression is increased in models of diabetic kidney disease [3,15•]. It is also known that ACE2 inhibition leads to worsening of albuminuria in diabetic mice [11–13,15•,16••,18–20,21••]. ACE2 may be involved in the hemodynamics of pregnancy [22]. In this report, we discuss conditions in which ACE2 expression is altered, and we review recent studies showing that Ang II receptor blockers, ACE inhibitors, and aldosterone antagonists modulate ACE2 expression.

Pharmacologic and Hormonal ACE2 Modulation

Studies in kidney and peritoneal cell lines have shown that cytokines can modulate ACE2 expression in vitro [23]. In Vero E6 cells, which are tubular epithelial cells from monkeys, interferon γ and interleukin 4 decreased ACE2 expression at the protein and gene levels [23]. In peritoneal macrophages from mice, aldactone (an aldosterone antagonist) decreased ACE2 enzymatic activity and gene expression [24••].

The effect of Ang II stimulation on ACE2 expression has been studied using animal experimental models and in vitro cultures. Gallagher et al. [25] showed that in cultured astrocytes, exposure to Ang II caused a reduction in neural ACE2 mRNA and protein, a response mediated by the Ang II (AT) 1 receptor. In concordance with this study, Ang II was able to up-regulate ACE and down-regulate

Table 1. Upregulation of ACE2 expression by renin-angiotensin-aldosterone blockers

Study	Drug	Species	Tissues/cells
Angiotensin II receptor blockers			
Igase et al. [32]	Olmesartan	Spontaneously hypertensive rats	Thoracic aorta
Whaley-Connell et al. [37]	Valsartan	Ren 2 transgenic rat	Kidney
Ferrario et al. [33]	Losartan	Lewis rats	Heart, renal cortex
Soler et al. [30]	Telmisartan	Mice	Renal vasculature
Angiotensin-converting enzyme blockers			
Ocaranza et al. [31]	Enalapril	Myocardial infarction rats	Heart
Jessup et al. [35•]	Lisinopril	Ren 2 transgenic rats	Heart
Ferrario et al. [33]	Lisinopril	Lewis rats	Kidney
Mineralocorticoid receptor blockers			
Keidar et al. [24••]	Spironolactone	Human	Macrophages
	Eplerenone	Mice	Heart

ACE—angiotensin-converting enzyme.

ACE2 in human kidney tubular cells. These effects were blocked by an AT1 receptor antagonist (losartan), but not by an AT2 receptor blocker (PD123319) [26•]. Furthermore, blockade of extracellular signal-regulated kinases 1/2 (ERK1/2) or p38 mitogen-activated protein (MAP) kinases by either specific inhibitors or a dominant-negative adenovirus abolished Ang II–induced ACE2 down-regulation in human kidney tubular cells [26•].

There is evidence that all-trans retinoic acid (at-RA) influences gene expression of RAS components. Zhong et al. [17] studied this relationship in SHR and WKY rats treated with daily intraperitoneal at-RA injection. They showed that ACE2 expression was markedly decreased in placebo-treated SHR when compared with WKY rats. In at-RA treated SHR, a significant up-regulation of ACE2 expression was observed in the heart and kidney [17]. Also, at-RA affects regulation of the stem cell marker octomer-4 (Oct-4) and eventually, cellular differentiation. Zulli et al. [27] showed that cells within atherosclerotic plaques of New Zealand White rabbits co-express ACE2 and the hematopoietic stem cell marker, CD34. Thus, at-RA treatment could affect plaque cellular biology via effects on cellular differentiation and blood pressure via its effect on ACE2 [27].

The hormone 17β-estradiol increased ACE2 protein and gene expression in ovariectomized female rats with the renal wrap model of hypertension. It also prevented glomerular and tubular injury in this experimental hypertensive model [28•]. In an experimental model of acute renal failure after lipopolysaccharide treatment (rat model of endotoxemia), Gupta et al. [29] showed that activated protein C (APC) injection modulated the RAS by reducing ACE and angiotensinogen. APC was also shown to increase ACE2 mRNA levels in the kidney. This experiment illustrates that the potential protective role of APC in the kidney might be mediated by enhanced renal ACE2 expression, which is consistent with the original proposition that ACE2 may be renoprotective [19].

Effect of Renin-Angiotensin-Aldosterone System Blockade on ACE2 Expression

Some antihypertensive drugs have been shown to increase ACE2 enzymatic activity and protein and gene expression in different species, tissues, and cells (Table 1) [24••,30–33].

In the heart, Ang II receptor blockers have been shown to increase ACE2 protein and gene expression in different models of experimental hypertension [34,35•,36]. In the model of myocardial infarction after left coronary artery ligation and in transgenic Ren-2 rats, the ACE inhibitors enalapril and lisinopril increased heart ACE2 expression [31,35•]. In the thoracic aorta of male SHR, ACE2 was increased in association with reversal of vascular hypertrophy in response to olmesartan treatment [32]. In a study by Whaley-Connell et al. [37] that examined glomerular filtration barrier injury in the Ren-2 transgenic rat, AT1 receptor blockade was associated with increased ACE2 expression. These changes were not observed in atenolol- or hydralazine-treated hypertensive rats [32]. Taken together, these findings suggest that ACE2 is regulated by AT1 receptors and may be involved in mediating the pressure-independent vascular remodeling effects of Ang II blockers [32].

Cardiac Ang II concentration and activity of MAP kinases were markedly increased in response to pressure overload in mice lacking ACE2 ($Ace2^{-/y}$ mice). Administration of candesartan, an AT1 receptor blocker, attenuated the hypertrophic response and suppressed the activation of MAP kinases in $Ace2^{-/y}$ mice [38•]. These results suggest that ACE2 plays an important role in regulating the hypertrophic response to pressure overload mediated by Ang II.

In a study by Oudit et al. [39•], *Ace2*$^{-/y}$ mutant mice developed a progressive, age-dependent, dilated cardiomyopathy with increased oxidative stress, neutrophilic infiltration, inflammatory cytokine and collagenase levels, MAP kinase activation, and pathologic hypertrophy. The AT1 receptor blocker irbesartan prevented the dilated cardiomyopathy in aged *Ace2*$^{-/y}$ mutant mice. This confirms the critical role of Ang II–mediated stimulation of AT1 receptors [39•].

In the kidney, both lisinopril and losartan increased ACE2 enzymatic activity in the renal cortex of adult Lewis rats [33]. Our laboratory showed that telmisartan increases ACE2 protein expression in the renal vasculature [30]. It is unknown if this action is related, in part, to the well-known peroxisome proliferator–activated receptor (PPAR) effect of telmisartan [40]. The PPARs are members of the nuclear receptor superfamily of ligand-activated transcription factors. In particular, PPAR-γ plays a critical role in regulating carbohydrate and lipid metabolisms. PPAR-γ ligands have modest antihypertensive effects related to their ability to promote peripheral vasodilation, improve insulin sensitivity, and decrease the risk for atherosclerosis [41]. The Ang II receptor blocker telmisartan is structurally similar to a PPAR-γ agonist. In fact, telmisartan treatment in vitro augmented PPAR-γ activity. Recently Kobayashi et al. [42] showed that in Dahl salt-sensitive hypertensive rats, telmisartan stimulates nitric oxide production through PPAR-γ and the Rho-kinase pathway. It also ameliorated cardiac hypertrophy and cardiovascular remodeling. A direct effect of PPARs on ACE2 expression has not been studied, but they could function in synergism with Ang II receptor blockers.

Aldosterone antagonists (eg, spironolactone and eplerenone) have been shown to increase ACE2 enzymatic activity in macrophages from humans and mice [24••]. Spironolactone treatment increased *ACE2* gene expression in human macrophages [24••]. Eplerenone treatment, on the other hand, increased ACE2 activity and decreased ACE activity in hearts from Balb/C mice as compared with vehicle-treated mice [24••]. This suggests that aldosterone inhibits ACE2, but the mechanism needs to be elucidated.

ACE2 Inhibitors and Activators

Although the foregoing studies suggest that indirect ways exist to influence ACE2 activity pharmacologically, namely by using agents that affect the RAS, there is an interest in exploring and developing agents that are primarily inhibitors or activators of ACE2. An ACE2 inhibitor developed by Millennium Pharmaceuticals (Cambridge, MA), MLN-4760, has been used in murine studies [16••,43•]. Our laboratory has studied the effect of the pharmacologic-specific ACE2 inhibition by MLN-4760 administration in two experimental models of diabetic nephropathy [13•,16••]. Administration of this inhibitor for 16 weeks resulted in worsening albuminuria in db/db mice, and this was associated with increased glomerular expression of fibronectin [16••]. In streptozotocin (STZ)-treated mice, Soler et al. [13•] found increased albuminuria, glomerular mesangial expansion, and vascular thickness after MLN-4760 treatment. Our finding of increased albuminuria in two models of diabetic mice treated with an ACE2 inhibitor suggests a role of this enzyme in regulating Ang II–mediated glomerular permeability. In agreement with this, Tikellis et al. [43•] recently found that in STZ-treated mice, albuminuria was increased after MLN-4760 administration for 4 weeks. ACE2-knockout diabetic mice using STZ experienced a 5.2-fold increase in urinary albumin excretion when compared with untreated ACE2-knockout mice [43•]. Surprisingly, ACE2 inhibition was able to attenuate diabetes-associated changes in osteopontin expression and glomerular fibronectin accumulation [43•]. The dissociation between the effects on albuminuria and fibrogenesis after ACE2 inhibition is unexpected, and both findings are difficult to reconcile. Tikellis et al. [43•] suggested that the decrease in fibronectin deposition was possibly related to a decrease in renal ACE activity in animals treated with the selective ACE2 inhibitor. The decrease in ACE activity observed by these authors was previously reported in kidney cortex from STZ diabetic mice treated with MLN-4760 [13•]. However, it must be noted that glomeruli from diabetic mice treated with MLN-4760 had an increase in ACE expression [13•]. In contrast, ACE2 expression is decreased (not increased) in kidney cortex, which is mostly composed of proximal tubules [15•]. Another interesting finding from Tikellis et al. [43•] is that perindopril reduced renal cortical ACE2 activity in both control and diabetic animals. Although the interaction of ACE and ACE2 appears complex and is not fully understood, ACE2 protein and activity may be influenced by the level of ACE protein and activity, and vice versa.

Ongoing studies are intended to develop drugs that enhance ACE2 activity. Recently, Hernandez Prada et al. [44••], using structure-base screening, found a compound named xanthenone that enhances ACE2 activity. This compound caused considerable reductions in blood pressure, and a striking reversal of cardiac and renal fibrosis in the SHR model of hypertension [44••].

We have used recombinant ACE2 (r-ACE2) as a novel approach to increase Ang II metabolism and reduce Ang II–dependent hypertension [45••]. We showed that the increase in blood pressure associated with Ang II infusion was abolished in mice infused simultaneously with r-ACE2. Thus, the administration of enzymatically functional ACE2 abrogates Ang II–induced hypertension [45••].

Conclusions

ACE2 is an enzymatically active homologue of ACE that plays a significant role in maintaining a balanced status of the RAS. Several studies have shown that ACE2 is altered under pathologic conditions, and its inhibition by pharma-

cologic or genetic deletion has been shown to accelerate kidney and heart injury. Drugs based on RAS blockade —ACE inhibitors and AT1 receptor blockers—appear to increase ACE2 expression in the heart and the vasculature. This effect may contribute to their antihypertensive and cardiovascular protective action. New strategies aimed at new drug targets that are more effective in ACE2 amplification may provide a therapeutic approach to protect against cardiovascular disease, kidney disease, and hypertension.

Disclosures

No potential conflicts of interest relevant to this article were reported.

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Investigators developed a microplate-based fluorometric method for concurrent determination of ACE and ACE2 activity in tissue samples. Results showed that ACE2 expression is increased at the posttranscriptional level in kidney cortex from diabetic mice. The availability of an assay for concurrent measurement of ACE and ACE2 activity should be helpful in evaluating kidney-specific alterations.

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