Full Review

ACE2 alterations in kidney disease

María José Soler1,
Jan Wysocki2
and Daniel Batlle2

1Department of Nephrology, Hospital del Mar-Fundació IMIM,
Barcelona, Spain
2Division of Nephrology & Hypertension, Department of Medicine,
The Feinberg School of Medicine, Northwestern University, Chicago,
IL 60611, USA

Correspondence and offprint requests to: María José Soler; E-mail: Msoler@parcdesalutmar.cat

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ABSTRACT

Angiotensin-converting enzyme 2 (ACE2) is a monooxygenase that degrades angiotensin (Ang) II to Ang-(1–7). ACE2 is highly expressed within the kidneys, it is largely localized in tubular epithelial cells and less prominently in glomerular epithelial cells and in the renal vasculature. ACE2 activity has been shown to be altered in diabetic kidney disease, hypertensive renal disease and in different models of kidney injury. There is often a dissociation between tubular and glomerular ACE2 expression, particularly in diabetic kidney disease where ACE2 expression is increased at the tubular level but decreased at the glomerular level. In this review, we will discuss alterations in circulating and renal ACE2 recently described in different renal pathologies and disease models as well as their possible significance.

INTRODUCTION

Angiotensin-converting enzyme 2 (ACE2) is the only known homologue of ACE with enzymatic activity, which shares 42% sequence identity to the N- and C-terminal domains of somatic ACE [1, 2]. Whereas ACE degrades angiotensin (Ang) I into Ang II and Ang II into Ang 1–7 and degrades Ang I to Ang 1–9 [3]. Initially, ACE2 was thought to be restricted to the kidney, heart and testes [2, 3]. Subsequently, however, ACE2 was found in other organs, such as lungs, liver, central nervous system and placenta [4–9]. In mouse kidney, ACE2 is highly expressed [10, 11] and its activity is much higher than in mouse heart tissue [10]. ACE and ACE2 co-localize in the brush border of mouse proximal tubules [12]. In glomeruli, however, both enzymes are localized in distinct cell types [12]. Within the glomerulus, ACE2 is mainly present in glomerular epithelial cells and, to a lesser extent, in glomerular mesangial cells. Glomerular ACE, in contrast, is localized in endothelial cells [12]. In human kidneys, the pattern of ACE2 expression is similar to that of mouse kidneys [13]. In kidneys from healthy control subjects, Lely et al. [13] found ACE2 expression in tubular, glomerular visceral and parietal epithelial cells, as well as in vascular muscular smooth muscle cells and the endothelium of interlobular arteries.

The renin–Ang system plays a key role in the control of renal function [14, 15]. Several studies have examined ACE2 in diabetic kidney disease [10, 12, 14, 15] and in different models of kidney injury. In this review, we will discuss alterations in ACE2 expression and/or activity recently described in different renal pathologies and their possible significance. Given the opposing actions on Ang II levels, ACE has often been studied along with ACE2 [13, 16].

DIABETIC NEPHROPATHY

In kidney cortex from db/db mice and streptozotocin (STZ) diabetic mice, we found that ACE2 was increased, whereas ACE was markedly decreased [11] (Table 1). The differences in both enzymes were found at the protein and enzymatic activity level [10]. In glomeruli from db/db mice, in contrast, ACE2 is decreased, whereas ACE expression is increased at 8 weeks of age when glomerular lesions are barely detectable [12]. We also found this pattern in db/db at 32 weeks of age when glomerular lesions are more established [12, 27]. An increase in ACE was also found in glomeruli from STZ diabetic rats [28] and in the STZ-treated diabetic mice [15]. In STZ
diabetic rats, a 31% decrease in glomerular ACE2 activity was reported although it did not appear to reach statistical significance (P = 0.06) [29]. A recent study in the \( \text{db/db} \) mice has confirmed the findings of decreased ACE2 in glomeruli but increased in tubules [29, 30]. The majority of animal studies therefore indicate that diabetes is associated with downregulation of ACE2 in the glomeruli, whereas in kidney tubules ACE2 is clearly upregulated (Figure 1).

In concordance with these studies in animal models, kidney biopsy studies have demonstrated decreased ACE2 in glomeruli from patients with type 2 diabetes and nephropathy [24, 31]. For example, Mizuiri et al. [24] found decreased glomerular ACE2 expression and increased glomerular ACE expression in patients with type 2 diabetes and overt nephropathy. Reich et al. [31] also showed that ACE2 mRNA levels were decreased in the glomeruli of patients with diabetic nephropathy (DN) compared with non-diabetic healthy control subjects. These investigators found that ACE2 expression was relatively low in human glomeruli when compared with proximal tubule cells in both normal and diabetic kidney biopsies, and treatment with an ACE inhibitor did not affect ACE2 expression in the diabetic kidney. In addition, ACE expression was also increased in the glomeruli of patients with DN when compared with non-diabetic controls [31].

Table 1. ACE2 in kidney cortex in different kidney pathologies

<table>
<thead>
<tr>
<th>Kidney disease</th>
<th>Species</th>
<th>ACE2</th>
<th>Year</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetic Nephropathy</td>
<td>( \text{db/db} ) Mice*</td>
<td>Increased</td>
<td>2006</td>
<td>[11, 12]</td>
</tr>
<tr>
<td></td>
<td>STZ mice</td>
<td>Increased</td>
<td>2008</td>
<td></td>
</tr>
<tr>
<td>Hypertensive renal disease</td>
<td>SHR rats</td>
<td>Decreased</td>
<td>2002</td>
<td>[1]</td>
</tr>
<tr>
<td></td>
<td>2KIC hypertensive rats</td>
<td>Decreased</td>
<td>2011</td>
<td>[18]</td>
</tr>
<tr>
<td></td>
<td>HS Obese Zucker rats</td>
<td>Decreased</td>
<td>2013</td>
<td>[19]</td>
</tr>
<tr>
<td>Primary glomerulopathies</td>
<td>Human, IgA nephropathy without RAS blockade*</td>
<td>Decreased</td>
<td>2011</td>
<td>[20]</td>
</tr>
<tr>
<td>Subtotal nephrectomy</td>
<td>Rats</td>
<td>Decreased</td>
<td>2010</td>
<td>[21]</td>
</tr>
<tr>
<td>Ischemia-reperfusion model</td>
<td>Wistar rats</td>
<td>Decreased</td>
<td>2010</td>
<td>[22]</td>
</tr>
<tr>
<td>Shock induced renal injury</td>
<td>Rats</td>
<td>Decreased</td>
<td>2007</td>
<td>[23]</td>
</tr>
</tbody>
</table>

\*In glomeruli, ACE2 expression by immunostaining has been found to be decreased [24–26].
The changes in ACE and ACE2 can be expressed as the ACE/ACE2 ratio. In pathological states, such as in diabetes-related kidney disease, ACE and ACE2 often go in opposite directions, and the ACE/ACE2 ratio is a convenient way to reflect this altered pattern. The ACE/ACE2 ratio correlated positively with the mean blood pressure (BP), fasting blood glucose, serum creatinine, proteinuria and hemoglobin A1c and inversely correlated with the estimated glomerular filtration rate (GFR) [32]. An increased ACE/ACE2 ratio in diabetic patients with overt nephropathy suggests renin-Ang system (RAS) activation, which may contribute to renal injury as a result of Ang II accumulation.

In summary, there appears to be a pattern of decreased glomerular ACE2 and increased ACE expression in diabetic kidney that may increase intraglomerular Ang II and contribute to the progression of DN. In contrast, in whole kidney cortex ACE is decreased and ACE2 increased [11, 12, 16] (Table 1).

Deficiency in ACE2 might be involved in the development of diabetic kidney disease [14, 15, 32, 33]. Pharmacological inhibition of ACE2 in STZ-induced diabetes in mice causes increased albuminuria and glomerular matrix expansion [15]. In Akita mice, a model of type 1 diabetes, deletion of the ACE2 gene has been reported to exacerbate albuminuria, associated with increased mesangial matrix deposition, glomerular basement membrane thickening and glomerulosclerosis, without significant changes in BP [33]. Interestingly, administration of human recombinant ACE2 (rACE2) to diabetic Akita mice significantly reduced albuminuria, and reduced the BP [14]. Similarly, overexpression of adenovirus carrying mouse ace2 gene to rats with STZ-induced diabetes was reported to diminish albuminuria and glomerulosclerosis, along with reducing systolic BP [34]. These studies, although expected, need further confirmation because it is not clear that sustained ACE2 amplification can be achieved when human recombinant ACE2 is given chronically to rodents who develop neutralizing antibodies [35]. Moreover, the authors did not address the question whether the effect of ACE2 was systemic or local within the kidney. The administration of recombinant ACE2 does not affect kidney ACE2 activity despite a 50-fold increase in plasma ACE2 activity [17, 36].

Using a glomerular-specific ACE2 transgenic mouse model, Nadarajah et al. [34] showed that overexpression of human ACE2 attenuates the development of nephropathy in mice with STZ-induced diabetes. This was shown by a transient delay in the development of albuminuria, independent of any effect on systolic BP, histological evidence of renal protection, namely an early reduction in mesangial expansion and attenuation of glomerular hypertrophy at 16 weeks. Partial preservation of expression of the podocyte proteins nephrin and synaptopodin, prevention of podocyte loss and reduction in cortical TGF-B1 expression were found at 8 weeks of age [32] (Figure 2). These results suggested that amplification of ACE2 locally within the glomerulus might provide a therapeutic strategy in the prevention and treatment of DN. Whether increasing solely the circulating ACE2 activity could also be beneficial, in our opinion, requires further studies.

Tikellis et al. reported a 2-fold increase in circulating ACE2 activity in STZ-induced diabetes of normotensive male mice using a synthetic substrate for ACE2 [17]. In agreement with this finding, Yamaleyeva et al. also found increased circulating ACE2 activity in early-onset diabetes after STZ administration in hypertensive mRen2.Lewis rats [36]. Recently, Soro-Paavonen et al. reported a significant increase in serum ACE2 activity (~20%) in male type 1 diabetics with micro- or macroalbuminuria when compared with controls or a diabetic cohort without albuminuria [37]. Although the source for circulating ACE2 is not currently known, ACE2 may be actively shed from the vascular surface through metalloproteinases such as ADAM 10 and ADAM 17 previously shown to release the enzyme from renal and pulmonary cells in culture [38]. In these cell culture systems, soluble ACE2 is shed from the plasma membrane, via cleavage at its ectodomain by the protease enzyme ADAM-17 [38, 39]. In addition, renal ADAM-17 is upregulated in Ang II-induced kidney injury [40] and in db/db diabetic mice [30], and the novo expression has been described in human kidney disease, in proximal tubules, podocytes and mesangial cells [41]. Zimpelmann et al. [42] demonstrated an increase in plasma Ang II levels in normotensive male rats following 2 weeks of diabetes. The higher levels of Ang II may reflect a coordinate increase in circulating ACE and ACE2 activity in early diabetes. The magnitude of the change in ACE was less than that of ACE2 in diabetic rats;
however, the overall activity level for ACE was markedly higher. These results suggest that the increase in circulating ACE2 might be a mechanism that may attenuate an increase in circulating Ang II, but the magnitude of the increase is still insufficient to prevent diabetes-related kidney injury arising from Ang II overactivity. Future studies that could increase circulating ACE2 more robustly could shed some light on whether amplifying serum ACE2 could have a beneficial impact on diabetic kidney injury [27].

Besides serum, ACE2 protein has also been found in the urine where it appears to be increased in diabetes as well [43, 44]. Increased urinary ACE2 activity in diabetic mice was found to be of tubular rather than glomerular origin and reflected an increase in enzymatically active ACE2 protein with two bands identified of molecular sizes of 110 and 75 kDa [44]. In a study in 50 patients with type 2 DN, Wang et al. found that there was a relationship between urinary mRNA expression of ACE2 and the degree of proteinuria, and to a lesser extent, estimated GFR and GFR decline rate nephropathy [45]. In addition, urinary ACE2 levels studied by western blotting were significantly higher in patients with DN than patients without DN [46]. Later studies by Park et al. also assessed urinary ACE2 concentration in 412 patients with type 2 diabetes [47]. They investigated the relationships of urinary ACE2 concentrations with glucose metabolism and urinary albumin excretion status. This study showed that urinary ACE2 levels appear to be elevated in subjects with type 2 diabetes mellitus and are associated with various metabolic parameters, such as HOMA-IR, fasting blood glucose, triglyceride, high-sensitivity C-reactive protein and serum creatinine. The authors also found that urinary ACE2 levels are associated with a higher risk of microalbuminuria, independent of various confounding factors [47]. The results of these studies suggest that urinary ACE2 may be a potential marker for diabetic kidney disease.

HYPERTENSION

In 2002, Crackower et al. showed that ACE2 was reduced at the gene and protein level in kidneys from three separate rat models of spontaneous and diet-induced hypertension [1]. In a subsequent study in adult spontaneous hypertensive (SHR) rats, kidney ACE2 expression was decreased when compared with Wistar-Kyoto (WKY) rats. Of note, SHR rats treated with retinoic acid displayed a significant upregulation of ACE2 expression in heart and kidney [48]. Tikellis et al. showed that the developmental pattern of ACE2 expression in the SHR kidney was altered before the onset of hypertension [49]. Over the course of renal development, ACE2 expression did not significantly change in the SHR kidney, whereas at the same time ACE2 expression increased in the control WKY rats [49]. Ferrario et al. studied the effect of angiotensin-converting enzyme inhibitors (ACEIs) and Ang II receptor blockers (ARBs) administration on ACE2 expression in another model of experimental hypertension [50]. In this study, renin transgenic hypertensive rats (mRen2)27 were crossed with Lewis normotensive rats, creating the Lew.Tg (mRen2) congenic strain [50]. As expected, in the Lew.Tg (mRen2) rats, decreasing Ang II activity either by reducing its generation or by preventing the ligand from binding to the Ang II type 1 receptor, normalized BP [50]. In addition, however, both ACEI and ARB administration increased ACE2 gene expression and protein activity in renal cortical tissue from Lew.Tg (mRen2) rats [50].

In mice on the C57BL/6 genetic background, ACE2 deficiency was associated with a modest increase in BP, whereas the absence of ACE2 had no effect on baseline BPs in 129/SvEv mice [51]. After Ang II challenge, however, plasma concentrations of Ang II were almost 3-fold higher in ACE2-deficient mice than in controls and, moreover, BPs were substantially higher in the ACE2-deficient mice than in wild type [51]. In ACE2-deficient mice infused with an acute load of Ang II, an increased accumulation of Ang II in the kidney was documented [17, 36]. Thus, ACE2 deficiency can worsen hypertension under conditions of Ang II excess. Interestingly, Ang II was able to downregulate ACE2 in human kidney tubular cells [19]. These effects were blocked by an Ang II type 1 (AT1) receptor antagonist (losartan), but not by an AT2 receptor blocker [52]. Furthermore, blockade of extracellular signal-regulated kinases 1/2 or p38 mitogen-activated protein kinases by either specific inhibitors or a dominant-negative adenovirus, abolished Ang II-induced ACE2 downregulation in human kidney tubular cells [52].

In the model of 2 kidney 1 clip (2K1C) hypertensive rats, Prieto et al. [53] demonstrated that ACE2 mRNA levels and activity are reduced in both kidneys (clipped and non-clipped) as well as decreased specific immunoreactivity of ACE2 in the kidneys. In contrast, ACE mRNA levels and enzyme activity are increased in both kidneys of 2K1C hypertensive rats [18]. The combined effect of increased ACE leading to increased Ang II formation, coupled with decreased ACE2, leading to diminished Ang II degradation to Ang1–7, would be to increase Ang II, as well as to reduce Ang 1–7 levels.

Recently, the effect of high salt (HS) intake on RAS regulation in obese Zucker rats has been studied. In the kidney cortex of obese rats, HS intake reduced cortical expression of the ACE2-ANG II-type 2 receptor-Mas receptor axis and a concomitant increase in Ang II levels [19]. The decrease in renal ACE2 expression in obese rats on HS may have been due to a pronounced increase in Ang II levels/AT1R function in the kidneys of these animals. Similarly, increased Ang II levels/AT1R function observed in the study might have caused a reduction in AT2R expression in obese rats on HS [19]. Overall, it appears that the higher levels of Ang II may be the primary trigger leading to changes in other RAS components and shifting the balance towards pro-hypertensive mechanisms. In addition, the significant reduction in AT2R and MasR expression in obese animals may affect other cellular changes such as cell growth and apoptosis, which may have more than the opposing hemodynamic effect of AT1 receptor. Bernardi et al. [53] also showed that a high dietary content of salt significantly increases glomerular ACE/ACE2 mRNA and protein ratios, which may regulate the degree of glomerular oxidative stress and therefore may be considered one of the mediators of salt-driven kidney damage. In this study, the effect of salt on renal oxidative stress and ACE/ACE2 modulation was independent of BP increase and was not due to
changes in circulating aldosterone [53]. The effect of salt on renal oxidative stress and ACE/ACE2 modulation was independent of BP. These results are consistent with reports that a high-salt diet produced fibrosis in the kidney of both normotensive and hypertensive rats [54]. Moreover, since there is an inverse relationship between sodium intake and serum aldosterone, and aldosterone may downregulate ACE2 in the kidney [55], circulating aldosterone was measured to exclude any influence of this hormone on renal RAS. Both 1.2 and 8.2% salt diets fed rats demonstrated a non-significant reduction of circulating aldosterone, suggesting that the modulation of ACE/ACE2 ratio by the salt content of a diet is independent of this hormone [53].

The availability of mouse recombinant ACE2 with well-defined properties could provide a valuable tool to examine the potential therapeutic benefit of increasing ACE2 activity and fostering the formation of Ang-(1–7) from Ang II in mouse models of hypertension [36]. This will be especially useful for studying chronic disease mouse models where recombinant ACE2 is not anticipated to produce neutralizing antibodies [36]. Similar to human recombinant ACE2 [35], the administration of murine recombinant ACE2 markedly attenuated Ang II-induced hypertension and rapidly decreased plasma Ang II levels [17, 36, 56]. Moreover, the prevention of Ang II-induced hypertension by mouse rACE2 was completely obliterated by a specific ACE2 inhibitor MLN-4760 [56]. Interestingly, these studies also disclosed some differences between human and mouse rACE2. For instance, not all compounds that have been known to inhibit human rACE2 also show an inhibitory effect on the activity of mouse rACE2. MLN-4760 inhibits both human and murine ACE2, whereas DX600 inhibits human ACE2 preferentially. Mouse rACE2 effectively degrades Ang II (1–8) forming Ang-(1–7), whereas it has no effect on the formation of Ang-(1–9) from Ang I (1–10) [56]. More recently, in the spontaneously hypertensive rat model, recombinant human ACE2 delivered over a 14-day period partly corrected the hypertension, the NADPH oxidase activation and the increased superoxide generation in the heart, kidney and blood vessels [57]. Treatment with human rACE2 inhibited Ang II-mediated phosphorylation of the myocardial extracellular signal regulated kinase 1/2 pathway in WKY rats, with congruent results seen in SHR hearts. It is unclear to us whether such effects could be attributed solely to augmented ACE2 activity because the prolonged use of human rACE2 given to rats is expected to induce immunogenicity and the development of antibodies that neutralize ACE2 activity.

In renal biopsies from humans, the ratio of ACE to ACE2 gene expression was significantly higher in subjects with hypertension than in subjects without hypertension [58]. Keidar et al. [59] found that hypertensive patients had higher ACE2 activity in monocyte-derived macrophages when compared with hypertensive subjects. In concordance, Rice et al. in a large cohort detected ACE2 activity in only 7.5% of the subjects [60]. Interestingly, subjects with detectable ACE2 were older and had a higher prevalence of cardiovascular disease, diabetes and hypertension, suggesting that ACE2 may be upregulated in subjects with cardiovascular disease to reduce Ang II and its adverse effects [60].

**PRIMARY GLOMERULOPATHIES**

In human renal biopsies, Lely et al. [13] found increased ACE2 protein expression in glomerular visceral and parietal epithelium when compared with normal kidneys. In addition, they found ACE2 neoexpression in the glomerular endothelium and mesangium from primary renal disease patients when compared with samples from normal kidneys [13]. In membranous glomerulopathy, the number of patients allowed for comparison between subjects treated with ACE inhibition versus those without ACE inhibition; however, no differences in ACE2 expression could be detected between these two groups. Furthermore, ACE2 expression was not related to the severity of renal involvement (as estimated from serum creatinine or proteinuria) across the different diagnostic groups. No relationship between ACE2 expression, gender or age could be detected [13].

Mizuiri et al. studied ACE and ACE2 expression in kidneys from glomerular disease, 13 primary membranous nephropathy, 14 minimal change nephrotic syndrome and 20 healthy controls (kidney transplant donors). Subjects treated with either ACEI or ARB were excluded from this study [20]. Reduced ACE2 expression and increased ACE expression were found in glomeruli from patients with IgA nephropathy compared with healthy controls, although the shift in ACE2 mRNA was not statistically significant [20]. The differences between the Lely and Mizuiri studies are not clear but perhaps could be ascribed, in part, to RAS blockade in subjects from the first mentioned study. Thus, in patients with IgA nephropathy without RAS blockade IgA nephropathy is associated with increased glomerular ACE expression and decreased ACE2 expression in both the tubulointerstitium and glomeruli, as in DN. Increased ACE and decreased ACE2 expression may represent a generalized response to glomerular kidney injury.

Reich et al. studied ACE2 and ACE gene expression in kidney biopsies from patients with focal segmental glomerulosclerosis (FSGS). They did not find differences between ACE2 and ACE mRNA levels in the kidney biopsies of subjects with FSGS or controls [31]. Interestingly, blockade of the RAS (with ACE inhibitor/ARB) in subjects with FSGS was associated with an increase in the tubular expression of ACE2, raising the possibility that ACE inhibitor/ARB use may upregulate ACE2 levels in subjects with FSGS [31].

**OBSTRUCTIVE NEPHROPATHY**

In a recent paper, Liu et al. studied the effect of ACE2 deletion in a model with a well-established chronic kidney disease (without underlying hypertensive and diabetic conditions), the unilateral ureteral obstructive (UUO) nephropathy. They found that loss of Ace2 in this mouse model of nephropathy enhanced renal fibrosis and inflammation which were associated with an increase in the intrarenal TGF-β/Smad2/3 and NF-kB signaling pathways [61]. Obstructed kidneys from ACE2KO mice had significantly increased kidney Ang II and decreased kidney Ang-(1–7) levels, but no change in plasma...
levels of these Ang peptides were observed. Consistent with these findings, intrarenal Ang II signaling (AT1-ERK1/2 mitogen-activated protein kinase) was also increased in ACE2KO-UUO mice. Whether these pro-fibrotic actions were attributable to an increase in intrarenal Ang II, decrease in Ang-(1–7) or both was not examined in this study. However, the role of the Mas receptor in renal fibrosis induced by UUO was examined in a separate study by Esteban et al. in Mas receptor knockout mice (on the same C57Bl/6 genetic background). Interestingly, Mas KO showed less injury than wild type with UUO, as evidenced by decreased intrarenal NF-κB levels, matrix deposition, apoptosis and inflammatory cell infiltration when compared with obstructed kidneys from WT mice with UUO [62]. This suggested that Ang-(1–7) is detrimental to the kidneys in the course of unilateral ureteral obstruction. Indeed, WT mice that were infused with Ang-(1–7) had more severe renal lesions and fibrotic responses compared with mice that did not receive Ang-(1–7). These deleterious effects could not have originated from activation of AT1A, AT1B and AT2 receptors by Ang-(1–7) since the respective Ang II receptor mutant mice exhibited similar responses to those seen in WT mice. Altogether, the finding that ACE2 controls, in part, Ang II degradation locally in a mouse model of UUO nephropathy is clinically relevant. It implies that ACE2 acts as a local regulator for renal protection against the endogenous Ang II-mediated injury independent of hypertension [61]. Thus, ACE2 appears to be an essential regulator in maintaining the balance between Ang II generation and Ang II degradation locally within the kidney regardless of systemic disease conditions.

**ISCHEMIA-REPERFUSION MODEL AND KIDNEY TRANSPLANT PATIENTS**

Renal ischemia appears to change or to modulate the profile of components of the RAS. Da Silveira et al. [22] examined the renal profile of Ang-(1–7), ACE2 and the Mas receptor in renal ischemia/reperfusion (I/R) and compared them with that of Ang II, ACE and the AT1 receptor. Male Wistar rats were submitted to left nephrectomy and ischemia (45 min) followed by reperfusion (2 or 4 h) in the right kidney. At 4 h of reperfusion, renal Ang II was increased and renal Ang-(1–7) was decreased substantially, although plasma levels of both Angs were unchanged. In addition, renal I/R decreased the renal mRNA expression of renin, AT1 receptors and ACE2. At 2 and 4 h of reperfusion, renal ACE activity was reduced [22]. On the other hand, renal expression of the Mas receptor was greatly increased at 4 h of reperfusion, which was confirmed by immunohistochemical and western blot analysis. In conclusion, increased renal expression of the Mas receptor associated with changes in the RAS-related peptidases support an important role for the ACE2–Ang-(1–7)–Mas axis in acute kidney injury (AKI) [22]. These findings clearly show the complexity of the interactions between both arms of the RAS axis in AKI.

Recently, Soler et al. studied circulating ACE2 activity in kidney transplant patients. They found that circulating enzymatic ACE2 activity could be measured in KT patients and correlates with graft function, glycosylated hemoglobin and liver function parameters [63]. These findings suggest that measurement of serum ACE2 may be used as a non-invasive marker to understand the role of RAS in KT patients. Moreover, urinary ACE2 activity and ACE2 protein are increased in kidney transplant recipients, compared to healthy control subjects, and the presence of diabetes strongly associates with urinary ACE2 levels in the patient population [64]. These studies further suggest that ACE2 may be shed into the urine and serum in transplant recipients, and could represent a marker to assess the role of the kidney RAS in these patients.

**SUBTOTAL NEPHRECTOMY**

Velkoska et al. studied the potential role of ACE2 in rats with acute kidney injury induced by STNx (subtotal nephrectomy). In this model, nephrons are lost through surgical ablation and the remaining nephrons undergo physiological changes, resulting in hypertrophy and hyperfiltration to compensate for the nephron loss [21]. Early after surgery, rats develop hypertension, polyuria and proteinuria. Over more prolonged time periods, glomerulosclerosis and tubular atrophy further accelerated nephron loss, leading to chronic renal disease (CKD) associated with a decrease in creatinine clearance and tubulointerstitial fibrosis [21]. They found that an acute reduction in renal mass by STNx (10 days after) increased circulating levels of the enzyme ACE2 and leads to a 50% decrease in renal ACE2 enzymatic activity (Table 1), which was accompanied by a significant increase in renal ACE activity of the same order. After administration of ramipril to STNx rats, the renal expression of ACE changed in the opposite direction to ACE2, so that as ACE decreased ACE2 activity was enhanced [21]. These results are consistent with the notion that ACE2 may be playing a protective role in the diseased kidney. As ACE2 both degrades Ang II and generates the vasodilator and anti-fibrotic peptide Ang-(1–7), a decrease in renal ACE2 activity, as observed in the present study, has the potential to contribute to the progression of renal injury. Support for this hypothesis comes from a recent study in the mas receptor-knockout mouse, where a lack of Ang-(1–7) action at the renal level caused hyperfiltration, proteinuria and a tendency toward glomerulosclerosis [65]. Further studies from the same group demonstrated that the decrease in kidney ACE2 previously reported after acute STNx was still present at 4 weeks and is likely to contribute to progression of renal disease. In contrast, the early elevation in cardiac ACE2 after STNx did not persist long term and again may contribute to ongoing cardiac dysfunction as renal disease progresses [66]. The main finding of this study was the differential regulation of renal and cardiac ACE2 expression in rats with CKD secondary to renal mass reduction by STNx [66]. ACE inhibition in STNx only partly ameliorated the cardiac and renal complications of renal failure, despite potent antihypertensive and tissue (kidney and cardiac) ACE inhibitory effects. Thus, in rats with CKD it appears that renal ACE2 deficiency and lack of activation of cardiac ACE2 may attenuate the beneficial effects of ACE...
inhibition and contribute to the progression of disease. Adjunctive therapies to ACE inhibition alone, such as strategies that lead to increases in ACE2 activity over and above levels seen in normal physiology, may be needed to combat the cardio-renal complications of CKD. In agreement with these findings, Dilauro et al. demonstrated that kidney ACE2 is downregulated in early CKD in the 5/6 nephrectomy in FVB mice. In addition, inhibition of ACE2 increases kidney Ang II levels and albuminuria via an AT1 receptor-dependent mechanism. Their data also indicate that administration of ANG-(1–7) to 5/6 Nx mice normalizes kidney ACE2 expression and levels of Ang II in kidney and plasma, yet does not affect BP, urinary albumin excretion, or FITC-inulin clearance. Indeed, the results showed that Ang-(1–7) increases the relative mesangial area compared with Sham mice. Losartan administration also normalized kidney cortical ACE2 expression [67].

SHOCK-INDUCED RENAL INJURY

In a model of acute renal failure (ARF) and endotoxemia after lipopolysaccharide administration, Gupta et al. showed decreased ACE2 gene expression in kidney tissue when compared with kidneys from control rats [23]. Interestingly, activated protein C injection in this model of ARF modulated the RAS by reducing ACE, angiotensinogen and increasing ACE2 mRNA levels in the kidney [23]. Recently, a study was published that aimed to test the effect of RAS disequilibrium on tourniquet-induced kidney injury. This study showed that ACE and ACE2 expressions changed inversely in kidneys of mice with tourniquet-induced shock, accompanied by enhancement of renal oxidative stress and deterioration of renal function and structure [68]. Moreover, circulating Ang II/Ang (1–7) imbalance also developed in limb ischemia–reperfusion (LIR) mice in accordance with changes of renal ACE and ACE2 expression, but the renal tissue Ang (1–7) concentration in contradiction with changes of local ACE and ACE2 expression also increased as renal tissue Ang II concentration increased [68]. Interestingly, they observed that ACE/ACE2 expression imbalance in local tissue was in accordance with changes in circulating Ang II/Ang (1–7). However, the renal tissue Ang (1–7) concentration in contradiction with changes in local ACE and ACE2 expression also increased as renal tissue Ang II concentration increased [68]. These results suggested that circulating RAS was closely associated with kidney-derived ACE and ACE2, and the local renal RAS was independent of the circulating RAS. In addition, ACE2 knock-out exacerbated while its overexpression relieved the renal ACE/ACE2 and ultimately serum Ang II/Ang (1–7) imbalance during shock, resulting in further enhancement or abatement, respectively, of renal injury and mortality. These findings suggest that circulating and local renal tissue RAS may have different activated mechanisms and roles in LIR-induced renal injury, and disequilibrium of the two pathways in RAS may play an important role in this state [68]. Thus, targeting ACE2-Ang (1–7) pathway may be a potential treatment approach for shock-induced renal injury.

CHRONIC KIDNEY DISEASE

Recently, Roberts et al. showed that among patients with CKD plasma ACE2 activity is lower in those undergoing hemodialysis for end-stage renal disease (ESRD) when compared with pre-dialysis patients with CKD or renal transplant patients [69]. When compared with historic samples from healthy subjects, however, all CKD groups examined, i.e. pre-dialysis, transplant patients and even subjects on dialysis, seemed to have increased levels of plasma ACE2 activity. Statistical comparisons with healthy controls, however, could not be done because samples from healthy individuals were not assayed concurrently with those in this study [69]. Accordingly, to demonstrate that ACE2 in plasma is indeed altered in CKD patients, further studies that include contemporary measurements from healthy control subjects are needed.

Of note, in patients undergoing dialysis, a significant difference in the level of ACE2 plasma activity was demonstrated between males and females [69]. Although the ACE2 gene is located on the X-chromosome, the level of ACE2 activity was higher in males, which confirms the work of others who showed that serum ACE2 activity is sex dependent, with higher levels in males compared with females [37]. The reduction in plasma ACE2 activity reported in ESRD patients treated by dialysis, particularly in female subjects, could limit Ang II degradation leading to increased levels of this peptide which could contribute to the high prevalence of hypertension and cardiovascular morbidity that affects the dialysis population [70].

The study by Roberts et al. [69] suggests that while plasma ACE2 activity may be increased in CKD patients by the time that ESRD is reached and dialysis initiated a relative deficiency in plasma ACE2 activity ensues [70]. The initial increase in the ACE2 activity with CKD could be compensatory and help dispose of Ang II by enhancing its degradation. As ESRD develops, however, the levels of this peptide would be augmented, thereby predisposing to hypertension and other cardiovascular morbidity [70]. Indeed, a very high prevalence of hypertension [71] and cardiovascular morbidity [72, 73] is well known to afflict the dialysis population regardless of age and gender.

The significance of plasma ACE2 activity, however, is not totally clear, since ACE2 is mainly a tissue enzyme and its levels in the circulation, unlike the levels of ACE, are relatively low [70]. Interestingly, in pathological states in humans, such as ischemic heart disease, heart failure, and diabetes accompanied by vascular complications as well as in rodent models of diabetes circulating ACE2 activity is augmented [37, 74, 75]. The presence of a small molecular endogenous inhibitor of ACE2 in human plasma has been reported [76]. Hemodialysis itself could potentially alter the levels of the ACE2 inhibitor in plasma owing to its small molecular size. Removal of the inhibitor during the dialysis procedure, however, could only increase plasma ACE2 activity, which is the opposite of what was observed by Roberts et al. in their dialysis patients [71].
If the plasma ACE2 inhibitor indeed does not play a role in dialysis patients, the question that remains is what causes the observed reduction in plasma ACE2 activity in such patients. The source of circulating ACE2 in healthy individuals and CKD patients is not clear since this enzyme is not present in healthy endothelium and therefore cannot be shed into the circulation. Release from the kidneys is a possibility [70] since ACE2 activity is very high in the kidney but the source of circulating ACE2 remains to be determined.

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