27. Pula G, Mayr U, Evans C *et al.* Proteomics identifies thymidine phosphorylase as a key regulator of the angiogenic potential of colony-forming units and endothelial progenitor cell cultures. Circ Res 2009; 104: 32–40
28. Manon-Jensen T, Itoh Y, Couchman J. Proteoglycans in health and disease: the multiple roles of syndecan shedding. FEBS J 2010; 277: 3876–3889
29. Dimmeler S, Leri A. Aging and disease as modifiers of efficacy of cell therapy. Circ Res 2008; 102: 1319–1330
30. Ceccaldi R, Parmar K, Mouly E *et al.* Bone marrow failure in Fanconi anemia is triggered by an exacerbated p53/p21 DNA damage response that impairs hematopoietic stem and progenitor cells. Cell Stem Cell 2012; 11: 36–49
31. Chen J, Xavier S, Moskowitz-Kassai E *et al.* Cathepsin cleavage of sirtuin 1 in endothelial progenitor cells mediates stress-induced premature senescence. Am J Pathol 2012; 180: 973–983
32. Chen J, Li H, Addabbo F *et al.* Adoptive transfer of syngeneic bone marrow-derived cells in mice with obesity-induced diabetes: selenoorganic antioxidant ebselen restores stem cell competence. Am J Pathol 2009; 174: 701–711
33. Iglesias-Bartolome R, Gutkind S. Signaling circuitries controlling stem cell fate: to be or not to be. Curr Opin Cell Biol 2011; 23: 716–723
34. Williams S, Beronja S, Pasolli H *et al.* Asymmetric cell divisions promote Notch-dependent epidermal differentiation. Nature 2011; 470: 353–358
35. Ingber DE. Cellular mechanotransduction: putting all the pieces together again. FASEB J 2006; 20: 811–827
36. Zouani O, Chanceau C, Broillaaud B *et al.* Altered nanofeature size dictates stem cell differentiation. J Cell Sci 2012; 125: 1217–1224
37. Song M, Brady-Kalnay S, McBride S *et al.* Mapping the mechanome of live stem cells using a novel method to measure local strain fields in situ at the fluid-cell interface. PloS One 2012; 7: e43601
38. Wolfe R, Ahsan T. Shear stress during early embryonic stem cell differentiation promotes hematopoietic and endothelial phenotypes. Biotechnol Bioeng 2012; epub on 8 November
39. Egorova A, DeRuiter M, de Boer H *et al.* Endothelial colony-forming cells show a mature transcriptional response to shear stress. In vitro Cell Dev Biol 2012; 48: 21–29
40. Discher D, Mooney D, Zandstra P. Growth factors, matrices, and forces combine and control stem cells. Science 2009; 324: 1673–1677
41. Iglesias-Bartolome R, Patel V, Cotrim A *et al.* mTOR inhibition prevents epithelial stem cell senescence and protects from radiation-induced mucositis. Cell Stem Cell 2012; 11: 401–414
42. Wang G, Babylak S, Heber-Katz E *et al.* The effects of DNA methyltrasferase inhibitors and histone deacetylase inhibitors on digit regeneration in the mouse. Regen Med 2010; 5: 201–220

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FULL REVIEW

With blog commentary

ACE2 alterations in kidney disease

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ABSTRACT

Angiotensin-converting enzyme 2 (ACE2) is a monocarboxypeptidase 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, ACE2 cleaves 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 *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

Table 1. ACE2 in kidney cortex in different kidney pathologies

Kidney disease	Species	ACE2	Year	References
Diabetic Nephropathy	*db/db* Mice* STZ mice	Increased Increased	2006 2008	[11, 12] [17]
Hypertensive renal disease	SHR rats 2K1C hypertensive rats HS Obese Zucker rats	Decreased Decreased Decreased	2002 2011 2013	[1] [18] [19]
Primary glomerulopathies	Human, IgA nephropathy without RAS blockade*	Decreased	2011	[20]
Subtotal nephrectomy	Rats	Decreased	2010	[21]
Ischemia-reperfusion model	Wistar rats	Decreased	2010	[22]
Shock induced renal injury	Rats	Decreased	2007	[23]

*In glomeruli, ACE2 expression by immunostaining has been found to be decreased [24–26].

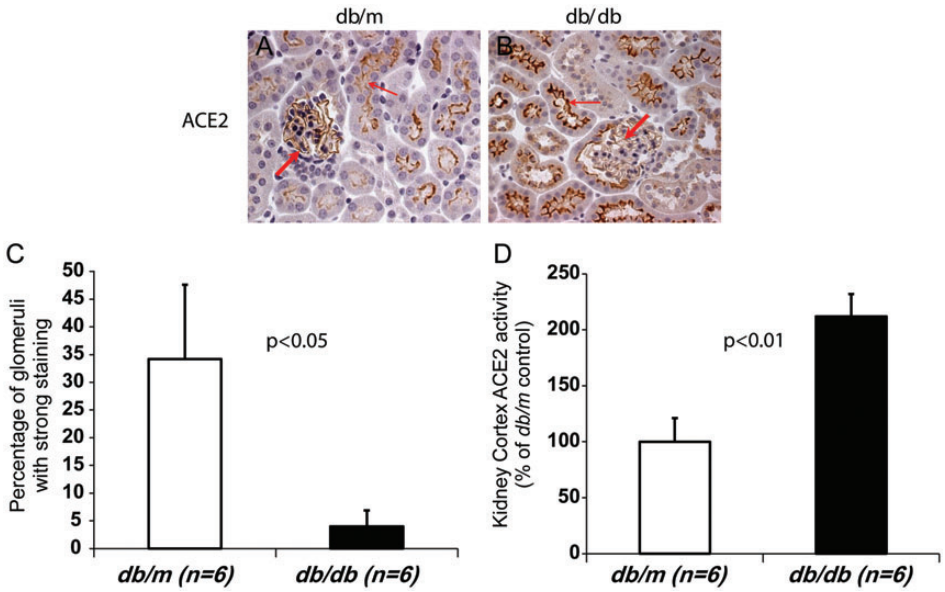

FIGURE 1: (Upper image) Immunohistochemistry of ACE2 (**A** and **B**) in kidney sections from 8-week-old female *db/m* (A) and *db/db* mice (B) showing an example of glomerular ACE2 staining. In *db/db*, ACE2 staining within the glomerular tuft (B, wide arrow) is less intense than in non-diabetic *db/m* (A, wide arrow). Unlike glomeruli, proximal tubules from *db/db* mice have more staining for ACE2 (B, narrow arrow) than proximal tubules from *db/m* (A, narrow arrow). (bar graphs) The percentage of strongly stained glomeruli for ACE2 in 8-week-old *db/m* (open bar; $n = 6$) and *db/db* mice (closed bar; $n = 6$) (**C**). Strong ACE2 staining is significantly decreased in glomeruli from diabetic mice in comparison with controls(C). In contrast, kidney cortex ACE2 activity is significantly higher in *db/db* mice when compared with *db/m* (**D**) likely reflecting the increase in ACE2 at the tubular level [compare with (A) and (B)] [adapted from [12] (Panels A, B and C) and [10] (Panel D)]

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].

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-β1 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.

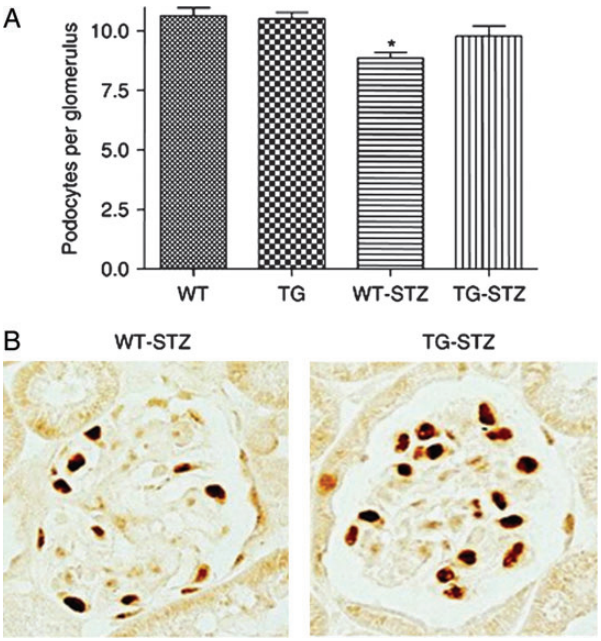

FIGURE 2: Podocyte number in WT, podocyte-spcific ACE2 transgenic (TG), wild-type streptozotocin-treated (WT-STZ), and podocyte-specific ACE2 transgenic STZ-treated (TG-STZ) mice at 16 weeks after STZ injection. (**A**) Graph shows average number of podocytes per glomerulus, as determined by counting of WT-1-positive nuclei in kidney sections. (**B**) Representative photomicrograph depicting glomerular WT-1 staining in glomeruli from WT-STZ versus TG-STZ mice. Original magnification ×400. Adapted by permission from Macmillan Publishers Ltd: Kidney International (ref 32), copyright (2012).

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 metallosecretases 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 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, Crackover *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 prehypertensive 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 afflicts 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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REFERENCES

1. Crackower MA, Sarao R, Oudit GY *et al.* Angiotensin-converting enzyme 2 is an essential regulator of heart function. Nature 2002; 417: 822–828
2. Donoghue M, Hsieh F, Baronas E *et al.* A novel angiotensin-converting enzyme-related carboxypeptidase (ACE2) converts angiotensin I to angiotensin 1–9. Circ Res 2000; 87: E1–E9
3. Tipnis SR, Hooper NM, Hyde R *et al.* A human homolog of angiotensin-converting enzyme. Cloning and functional expression as a captopril-insensitive carboxypeptidase. J Biol Chem 2000; 275: 33238–33243
4. Doobay MF, Talman LS, Obr TD *et al.* Differential expression of neuronal ACE2 in transgenic mice with overexpression of the brain renin-angiotensin system. Am J Physiol Regul Integr Comp Physiol 2007; 292: R373–R381
5. Hamming I, Timens W, Bulthuis ML *et al.* Tissue distribution of ACE2 protein, the functional receptor for SARS coronavirus. A first step in understanding SARS pathogenesis. J Pathol 2004; 203: 631–637
6. Imai Y, Kuba K, Rao S *et al.* Angiotensin-converting enzyme 2 protects from severe acute lung failure. Nature 2005; 436: 112–116
7. Paizis G, Tikellis C, Cooper ME *et al.* Chronic liver injury in rats and humans upregulates the novel enzyme angiotensin converting enzyme 2. Gut 2005; 54: 1790–1796
8. Valdes G, Neves LA, Anton L *et al.* Distribution of angiotensin-(1–7) and ACE2 in human placentas of normal and pathological pregnancies. Placenta 2006; 27: 200–207
9. Xie X, Chen J, Wang X *et al.* Age- and gender-related difference of ACE2 expression in rat lung. Life Sci. 2006; 78: 2166–2171
10. Wysocki J, Ye M, Soler MJ *et al.* ACE And ACE2 activity in diabetic mice. Diabetes 2006; 55: 2132–2139
11. Ye M, Wysocki J, Naaz P *et al.* Increased ACE 2 and decreased ACE protein in renal tubules from diabetic mice: a renoprotective combination? Hypertension 2004; 43: 1120–1125
12. Ye M, Wysocki J, William J *et al.* Glomerular localization and expression of angiotensin-converting enzyme 2 and angiotensin-

converting enzyme: implications for albuminuria in diabetes. J Am Soc Nephrol 2006; 17: 3067–3075
13. Lely AT, Hamming I, van Goor H *et al.* Renal ACE2 expression in human kidney disease. J. Pathol. 2004; 204: 587–593
14. Oudit GY, Liu GC, Zhong J *et al.* Human recombinant ACE2 reduces the progression of diabetic nephropathy. Diabetes 2010; 59: 529–538
15. Soler MJ, Wysocki J, Ye M *et al.* ACE2 Inhibition worsens glomerular injury in association with increased ACE expression in streptozotocin-induced diabetic mice. Kidney Int. 2007; 72: 614–623
16. Wysocki J, Soler M, Ye M *et al.* ACE2 is critically important for angiotensin II metabolism in podocytes. J. Am. Soc. Nephrol. 2006; 17: TH-PO877.293A
17. Tikellis C, Bialkowski K, Pete J *et al.* ACE2 deficiency modifies renoprotection afforded by ACE inhibition in experimental diabetes. Diabetes 2008; 57: 1018–1025
18. Prieto MC, Gonzalez-Villalobos RA, Botros FT *et al.* Reciprocal changes in renal ACE/ANG II and ACE2/ANG 1–7 are associated with enhanced collecting duct renin in Goldblatt hypertensive rats. Am J Physiol. Renal Physiol [Research Support, N.I.H., Extramural Research Support, Non-US Gov't]. 2011; 300: F749–55
19. Samuel P, Ali Q, Sabuhi R *et al.* High Na intake increases renal angiotensin II levels and reduces expression of the ACE2-AT(2) R-MasR axis in obese Zucker rats. Am J Physiol Renal Physiol [Research Support, N.I.H., Extramural Research Support, Non-US Govt]. 2012; 303: F412–F419
20. Mizuiri S, Hemmi H, Arita M *et al.* Increased ACE and decreased ACE2 expression in kidneys from patients with IgA nephropathy. Nephron Clin Pract [Research Support, Non-US Govt]. 2011; 117: c57–c66
21. Velkoska E, Dean RG, Burchill L *et al.* Reduction in renal ACE2 expression in subtotal nephrectomy in rats is ameliorated with ACE inhibition. Clin Sci [Research Support, Non-US Govt]. 2010; 118: 269–279
22. da Silveira KD, Pompermayer Bosco KS, Diniz LR *et al.* ACE2-angiotensin-(1–7)-Mas Axis in renal ischaemia/reperfusion injury in rats. Clin Sci [Research Support, Non-US Govt]. 2010; 119: 385–394
23. Gupta A, Rhodes GJ, Berg DT *et al.* Activated protein C ameliorates LPS-induced acute kidney injury and downregulates renal INOS and angiotensin 2. Am J Physiol Renal Physiol. 2007; 293: F245–F254
24. Mizuiri S, Hemmi H, Arita M *et al.* Expression of ACE and ACE2 in individuals with diabetic kidney disease and healthy controls. Am J Kidney Dis 2008; 51: 613–623
25. Batlle D, Soler MJ, Wysocki J. New aspects of the renin-angiotensin system: angiotensin-converting enzyme 2—a potential target for treatment of hypertension and diabetic nephropathy. Curr Opin Nephrol Hypertens 2008; 17: 250–257
26. Tikellis C, Johnston CI, Forbes JM *et al.* Characterization of renal angiotensin-converting enzyme 2 in diabetic nephropathy. Hypertension 2003; 41: 392–397
27. Batlle D, Wysocki J, Soler MJ *et al.* Angiotensin-converting enzyme 2: enhancing the degradation of angiotensin II as a potential therapy for diabetic nephropathy. Kidney Int 2012; 81: 520–528
28. Moon JY, Jeong KH, Lee SH *et al.* Renal ACE and ACE2 expression in early diabetic rats. Nephron Exp Nephrol 2008; 110: e8–e16
29. Leehey DJ, Singh AK, Bast JP *et al.* Glomerular renin angiotensin system in streptozotocin diabetic and Zucker diabetic fatty rats. Transl Res. 2008; 151: 208–216
30. Chodavarapu H, Grobe N, Somineni HK *et al.* Rosiglitazone treatment of type 2 diabetic db/db mice attenuates urinary albumin and angiotensin converting enzyme 2 excretion. PLoS ONE 2013; 8: e62833
31. Reich HN, Oudit GY, Penninger JM *et al.* Decreased glomerular and tubular expression of ACE2 in patients with type 2 diabetes and kidney disease. Kidney Int 2008; 74: 1610–1616
32. Nadarajah R, Milagres R, Dilauro M *et al.* Podocyte-specific overexpression of human angiotensin-converting enzyme 2 attenuates diabetic nephropathy in mice. Kidney Int [Research Support, N.I.H., Extramural Research Support, Non-U.S. Govt] 2012; 82: 292–303
33. Wong DW, Oudit GY, Reich H *et al.* Loss of angiotensin-converting enzyme-2 (Ace2) accelerates diabetic kidney injury. Am J Pathol 2007; 171: 438–451
34. Liu CX, Hu Q, Wang Y *et al.* Angiotensin-converting enzyme (ACE) 2 overexpression ameliorates glomerular injury in a rat model of diabetic nephropathy: a comparison with ACE inhibition. Mol Med [Research Support, Non-US Govt] 2011; 17: 59–69
35. Wysocki J, Ye M, Rodriguez E *et al.* Targeting the degradation of angiotensin II with recombinant angiotensin-converting enzyme 2: prevention of angiotensin II-dependent hypertension. Hypertension [Research Support, N.I.H., Extramural Research Support, Non-US Govt] 2010; 55: 90–98
36. Yamaleyeva LM, Gilliam-Davis S, Almeida I *et al.* Differential regulation of circulating and renal ACE2 and ACE in hypertensive mRen2.Lewis rats with early-onset diabetes. Am J Physiol Renal Physiol. [Research Support, N.I.H., Extramural Research Support, Non-US Govt]. 2012; 302: F1374–F1384
37. Soro-Paavonen A, Gordin D, Forsblom C *et al.* Circulating ACE2 activity is increased in patients with type 1 diabetes and vascular complications. J Hypertens 2012; 30: 375–383
38. Jia HP, Look DC, Tan P *et al.* Ectodomain shedding of angiotensin converting enzyme 2 in human airway epithelia. Am J Physiol Lung Cell Mol Physiol [Research Support, N.I.H., Extramural Research Support, Non-US Govt]. 2009; 297: L84–L96
39. Lambert DW, Yarski M, Warner FJ *et al.* Tumor necrosis factor-alpha convertase (ADAM17) mediates regulated ectodomain shedding of the severe-acute respiratory syndrome-coronavirus (SARS-CoV) receptor, angiotensin-converting enzyme-2 (ACE2). J. Biol Chem. [Research Support, Non-US Govt]. 2005; 280: 30113–9
40. Lautrette A, Li S, Alili R *et al.* Angiotensin II and EGF receptor cross-talk in chronic kidney diseases: a new therapeutic approach. Nat. Med [Comparative Study Research Support, N.I.H., Extramural Research Support, Non-U.S. Gov't Research Support, U.S. Gov't, P.H.S.] 2005; 11: 867–874
41. Melenhorst WB, Visser L, Timmer A *et al.* ADAM17 Upregulation in human renal disease: a role in modulating TGF-alpha availability? Am J Physiol Renal Physiol 2009; 297: F781–F790

42. Zimpelmann J, Kumar D, Levine DZ *et al.* Early diabetes mellitus stimulates proximal tubule renin mRNA expression in the rat. Kidney In. [Research Support, Non-U.S. Gov't] 2000; 58: 2320–2330
43. Wysocki J, Evora K, Ye M *et al.* Urinary excretion of ACE2—a biomarker of diabetic kidney disease? Am Soc Nephrol Renal Wk, November 16–21; Denver, CO. 2010
44. Wysocki J, Garcia-Halpin L, Ye M *et al.* Regulation of urinary ACE2 in diabetic mice. Am J Physiol Renal Physiol. 2013; Epub ahead of print June 12 (PMID: 23761674)
45. Wang G, Lai FM, Lai KB *et al.* Urinary mRNA expression of ACE and ACE2 in human type 2 diabetic nephropathy. Diabetologia 2008; 51: 1062–1067
46. Mizuiri S, Aoki T, Hemmi H *et al.* Urinary ACE2 in patients with CKD. Nephrology (Carlton) 2011 Apr 1
47. Park SE, Kim WJ, Park SW *et al.* High urinary ACE2 concentrations are associated with severity of glucose intolerance and microalbuminuria. Eur J Endocrinol 2013; 168: 203–210
48. Zhong JC, Huang DY, Yang YM *et al.* Upregulation of angiotensin-converting enzyme 2 by all-trans retinoic acid in spontaneously hypertensive rats. Hypertension 2004; 44: 907–912
49. Tikellis C, Cooper ME, Bialkowski K *et al.* Developmental expression of ACE2 in the SHR kidney: a role in hypertension? Kidney Int 2006; 70: 34–41
50. Jessup JA, Gallagher PE, Averill DB *et al.* Effect of angiotensin II blockade on a new congenic model of hypertension derived from transgenic Ren-2 rats. Am J Physiol Heart Circ Physiol 2006; 291: H2166–H2172
51. Gurley SB, Allred A, Le TH *et al.* Altered blood pressure responses and normal cardiac phenotype in ACE2-null mice. J Clin Invest. 2006; 116: 2218–2225
52. Koka V, Huang XR, Chung AC *et al.* Angiotensin II Up-Regulates Angiotensin I-Converting Enzyme (ACE), but Down-Regulates ACE2 via the AT1-ERK/p38 MAP Kinase Pathway. Am J Pathol 2008 Apr 10
53. Bernardi S, Toffoli B, Zennaro C *et al.* High-salt diet increases glomerular ACE/ACE2 ratio leading to oxidative stress and kidney damage. Nephrol Dial Transplant [Research Support, Non-US Gov't] 2012; 27: 1793–1800
54. Yu HC, Burrell LM, Black MJ *et al.* Salt induces myocardial and renal fibrosis in normotensive and hypertensive rats. Circulation [Research Support, Non-US Govt] 1998; 98: 2621–2628
55. Fukuda S, Horimai C, Harada K *et al.* Aldosterone-induced kidney injury is mediated by NFkappaB activation. Clin Exp Nephrol [Research Support, Non-US Govt] 2011; 15: 41–49
56. Ye M, Wysocki J, Gonzalez-Pacheco FR *et al.* Murine recombinant angiotensin-converting enzyme 2: effect on angiotensin II-dependent hypertension and distinctive angiotensin-converting enzyme 2 inhibitor characteristics on rodent and human angiotensin-converting enzyme 2. Hypertension [Comparative Study In Vitro Research Support, N.I.H., Extramural Research Support, Non-US Govt] 2012; 60: 730–740
57. Lo J, Patel VB, Wang Z *et al.* Angiotensin-converting enzyme 2 antagonizes angiotensin II-induced pressor response and NADPH oxidase activation in Wistar-Kyoto rats and spontaneously hypertensive rats. Exp Physiol 2013; 98: 109–122
58. Wakahara S, Konoshita T, Mizuno S *et al.* Synergistic expression of angiotensin-converting enzyme (ACE) and ACE2 in human renal tissue and confounding effects of hypertension on the ACE to ACE2 Ratio. Endocrinology 2007; 148: 2453–2457
59. Keidar S, Strizevsky A, Raz A *et al.* ACE2 Activity is increased in monocyte-derived macrophages from prehypertensive subjects. Nephrol Dial Transplant 2007; 22: 597–601
60. Rice GI, Jones AL, Grant PJ *et al.* Circulating activities of angiotensin-converting enzyme, its homolog, angiotensin-converting enzyme 2, and neprilysin in a family study. Hypertension 2006; 48: 914–920
61. Liu Z, Huang XR, Chen HY *et al.* Loss of angiotensin-converting enzyme 2 enhances TGF-beta/Smad-mediated renal fibrosis and NF-kappaB-driven renal inflammation in a mouse model of obstructive nephropathy. Lab Invest J Tech Methods Pathol [Research Support, Non-US Govt] 2012; 92: 650–661
62. Esteban V, Heringer-Walther S, Sterner-Kock A *et al.* Angiotensin-(1-7) and the g protein-coupled receptor MAS are key players in renal inflammation. PLoS ONE 2009; 4: e5406
63. Soler MJ, Riera M, Crespo M *et al.* Circulating angiotensin-converting enzyme 2 activity in kidney transplantation: a longitudinal Pilot Study. Nephron Clin Pract 2012; 121: c144–cc50
64. Xiao F, Hiremath S, Knoll G *et al.* Increased urinary angiotensin-converting enzyme 2 in renal transplant patients with diabetes. PLoS ONE [Research Support, Non-US Govt] 2012; 7: e37649
65. Pinheiro SV, Ferreira AJ, Kitten GT *et al.* Genetic deletion of the angiotensin-(1–7) receptor Mas leads to glomerular hyperfiltration and microalbuminuria. Kidney Int [Research Support, Non-US Govt] 2009; 75: 1184–1193
66. Burrell LM, Burchill L, Dean RG *et al.* Chronic kidney disease: cardiac and renal angiotensin-converting enzyme (ACE) 2 expression in rats after subtotal nephrectomy and the effect of ACE inhibition. Experim Physiol [Research Support, Non-US Gov't]. 2012; 97: 477–485
67. Dilauro M, Zimpelmann J, Robertson SJ *et al.* Effect of ACE2 and angiotensin-(1–7) in a mouse model of early chronic kidney disease. Am J Physiol Renal Physiol [Research Support, Non-US Govt] 2010; 298: F1523–F1532
68. Yang XH, Wang YH, Wang JJ *et al.* Role of angiotensin-converting enzyme (ACE and ACE2) imbalance on tourniquet-induced remote kidney injury in a mouse hindlimb ischemia-reperfusion model. Peptides [Research Support, Non-US Govt] 2012; 36: 60–70
69. Roberts MA, Velkoska E, Ierino FL *et al.* Angiotensin-converting enzyme 2 activity in patients with chronic kidney disease. Nephrol Dial Transplant. 2013; Epub ahead of print March 27 (PMID: 23535224)
70. Wysocki J, Batlle D. Reduced plasma ACE2 activity in dialysis patients: another piece in the conundrum of factors involved in hypertension and cardiovascular morbidity? Nephrol Dial Transplant. 2013; Epub ahead of print June 19 (PMID: 23787547)
71. Agarwal R, Nissenson AR, Batlle D *et al.* Prevalence, treatment, and control of hypertension in chronic hemodialysis patients in the United States. Am J Med [Clinical Trial Comparative Study Multicenter Study Randomized Controlled Trial] 2003; 115: 291–297
72. Martinez-Castelao A, Gorriz JL, Portoles JM *et al.* Baseline characteristics of patients with chronic kidney disease stage 3 and stage 4 in Spain: the MERENA observational cohort study. BMC Nephrol [Multicenter Study] 2011; 12: 53

73. Go AS, Chertow GM, Fan D *et al*. Chronic kidney disease and the risks of death, cardiovascular events, and hospitalization. N Engl J Med 2004; 351: 1296–1305
74. Ortiz-Perez JT, Riera M, Bosch X *et al*. Role of circulating angiotensin converting enzyme 2 in left ventricular remodeling following myocardial infarction: a prospective controlled study. PLoS ONE 2013; 8: e61695
75. Epelman S, Shrestha K, Troughton RW *et al*. Soluble angiotensin-converting enzyme 2 in human heart failure: relation with myocardial function and clinical outcomes. J Cardiac Fail 2009; 15: 565–571
76. Lew RA, Warner FJ, Hanchapola I *et al*. Angiotensin-converting enzyme 2 catalytic activity in human plasma is masked by an endogenous inhibitor. Exp Physiol 2008; 93: 685–693

NDT ERA-EDTA OLA has selected this publication for Blog commentary by its faculty in view of its quality and potential educational value.

Soler and colleagues review the relevance of changes in angiotensin converting enzyme-2 (ACE-2) to the physiology and pathophysiology of hypertension and kidney disease. They report changes in circulating and also renal ACE2 in a number of kidney disease postulating that a reduction in renal tissue ACE2 levels may prevent the degradation of angiotensin II (AII) to Ang(1-7), therefore potentiating the harmful effect of the former on the kidney whilst preventing the generation of the latter with its potential beneficial effects. They report a tissue ACE/ACE2 level increasing in most renal pathologies thus potentiating the generation of AII and reducing its breakdown.

Further, evidence suggests an important role of endogenous Ang(1-7) with the regulation by the brain of an endogenous antihypertensive axis consisting of ACE2 and Ang(1-7) (1). Such a counter-regulatory axis may modulate blood pressure and prevent renin-angiotensin-aldosterone system (RAAS) induced hypertension (1). Therefore reduced ACE2 activity in ESRD patients treated by dialysis may be yet another contributing factor to hypertension (2).

ACE2 is clearly the new player on the block of the RAAS and systemic hypertension in CKD. Its involvement along with Ang(1-7)-MAS1 and AngII/AngIII-ATR2 define the non-classical and protective pathways by contrast to the classical RAAS as defined by the ACE-AngII-AT1R axis that promotes vasoconstriction, sodium retention, and other mechanisms to maintain blood pressure, and increase oxidative stress. The classical RAAS pathway is also implicated in the pathogenesis of inflammation and renal fibrosis. Evidence also points to the non-classical RAS pathways as contributing to the therapeutic blockade of the classical RAAS system thus reduction of blood pressure and renal injury (3).

Life was relatively simple with the RAAS system and its inhibition, a better understanding of the non-classical RAS pathways open the way to new therapies that could be effective in reducing blood pressure and protecting damaged kidneys as well as preventing CKD progression.

The NDT ERA-EDTA OLA readers may be interested to learn more from the authors of this very interesting article about:

(1) Our understanding of the complexity of the RAS system may open the way to new therapies based on the activation of the ACE2/Ang(1-7)-MAS1 pathway. Are the authors aware of pharmacological interventions in clinical testing that would enhance ACE2 activity and therefore lower blood pressure?

(2) Is the protective effect of the non-classical ACE2/Ang(1-7)-MAS1 pathway entirely dependent on the degradation of AngII or is it also in part due to a direct vasodilatory and anti-oxidant vascular effect, perhaps mediated by the generation of nitric oxide and vasodilatory prostaglandins?

(3) Lower circulating ACE2 levels in female dialysis patients compared to males would suggest a gender and hormonal regulation of this enzyme and RAAS (4). Is this correlated with higher blood pressure levels in female patients treated by hemodialysis compared to males?

Prof Meguid El Nahas

FULL REVIEW

REFERENCES

1. Xue B, Zhang Z, Johnson RF, Guo F, Hay M, Johnson AK. Central Endogenous Angiotensin-(1-7) Protects against Aldosterone/NaCl-induced Hypertension in Female Rats. Am J Physiol Heart Circ Physiol. 2013 Jun 28. [Epub ahead of print]
2. Wysocki J, Batlle D. Reduced plasma ACE2 activity in dialysis patients: another piece in the conundrum of factors involved in hypertension cardiovascular morbidity? Nephrol Dial Transplant. 2013 Jun 19. [Epub ahead of print]
3. Chappell MC. Nonclassical Renin-Angiotensin system renal function. Compr Physiol. 2012 Oct 1;2(4):2733–52.
4. Roberts MA, Velkoska E, Ierino FL, Burrell LM. Angiotensin-converting enzyme 2 activity in patients with chronic kidney disease. Nephrol Dial Transplant. 2013 Mar 27. [Epub ahead of print]

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