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RAAS-mediated Redox effects in Chronic Kidney Disease

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Abstract

The renin-angiotensin-aldosterone-system (RAAS) is central to the pathogenesis of hypertension, cardiovascular and kidney disease. Emerging evidence support various pathways through which a local renal RAAS can affect kidney function, hypertension, and cardiovascular disease. A prominent mechanism appears to be loss of redox homeostasis and formation of excessive free radicals. Free radicals such as reactive oxygen species (ROS) are necessary in normal physiologic processes including development of nephrons, erythropoiesis and tubular sodium transport. However, loss of redox homeostasis contributes to pro-inflammatory and pro-fibrotic pathways in the kidney that in turn lead to reduced vascular compliance, podocyte pathology and proteinuria. Both blockade of the RAAS and oxidative stress produces salutary effects on hypertension and glomerular filtration barrier injury. Thus, the focus of current research is on understanding the pathophysiology of chronic kidney disease in the context of an elevated RAAS and unbalanced redox mechanisms.

Keywords

Kidney; Renin; Angiotensin; Aldosterone; Redox

INTRODUCTION

The renin-angiotensin-aldosterone system (RAAS) is a complex endocrine, paracrine and autocrine system that modulates the function of the kidney. Recent work has focused on dissecting the role of local RAAS in the kidney and evidence is emerging for its role in diabetic, hypertensive chronic kidney disease (CKD).^{1,2} Apart from traditional role in the kidney, the discovery of local robust RAAS expression has been defined in kidney function and pathology.

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As per the National Kidney Foundation (NKF)-Kidney Disease Outcomes Quality Initiative (KDOQI) consensus guidelines, CKD resulting from diabetes and hypertension remain the leading causes of renal morbidity and mortality.³ The basis for recognition of RAAS as the central player in the pathophysiology of CKD stems from several well-designed randomized clinical trials in Type 1 diabetes patients including the Collaborative Study Group (CSG) trial.⁴ In Type 2 diabetics, the Heart Outcomes Prevention Evaluation (HOPE), irbesartan on the development of diabetic nephropathy in patients with type 2 diabetes (IRMA2), MicroAlbuminuria Reduction With VALsartan (MARVAL), The Incipient to Overt: Angiotensin II Blocker, Telmisartan, Investigation on Type 2 Diabetic Nephropathy (INNOVATION), Irbesartan in Diabetic Nephropathy Trial (IDNT), Nephrologic Diabetes Complications Trial (BENEDICT), and Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan (RENAAL) studies, have demonstrated clear benefits from RAAS blockade with angiotensin converting enzyme (ACE) inhibitors and angiotensin receptor blockers (ARBs) (Table I).^{5–14}

The coexistence of RAAS activation and “oxidative stress” has generated extraordinary interest and is arguably the most important mechanism for diabetic CKD. Binding of angiotensin (Ang II) to the Ang type 1 receptor (AT₁R), stimulates a series of signaling cascades that are divided into G-protein and non-G-protein related signaling. An example of a G-protein-dependent pathway is activation of phospholipase C (PLC) with the subsequent production of inositol 1,4,5-phosphate (IP₃) and diacylglycerol (DAG). IP₃ then stimulates Ca²⁺-mediated activation of protein kinase C (PKC) that ultimately leads to activation of NAD(P)H oxidase enzyme complex and reactive oxygen species (ROS) generation. Non-G-protein-dependent pathways induced by Ang II include phosphorylation and activation of various tyrosine kinases. An example of this would be activation of epidermal growth factor receptor (EGFR) which then leads to activation of transcription factor c-Jun which could in turn induce ROS formation through NAD(P)H oxidase.

There is sufficient evidence to support the notion that Ang II promotes ROS formation via NAD(P)H oxidase activation by direct stimulatory effects (stimulation of the AT₁R); studies on inhibition of the RAAS have contributed to this evidence as well.^{15–21} Renal afferent arterioles when infused with Ang II at a slow pressor rate exhibit renal cortical NAD(P)H-stimulated superoxide (O₂^{•-}) generation and lipid peroxidation in addition to impaired acetylcholine (ACh)-induced endothelium-dependent relaxation and enhanced contractile responses to Ang II.¹⁷ The arterioles have a profound downregulation of the mRNA for AT₁R but an upregulation of the p22^{phox} component of NAD(P)H oxidase. In addition, Ang II infusion in rats led to increased expression of NAD(P)H oxidase subunits, an effect abrogated with blockade of the AT₁R.¹⁵ Moreover, administration of AT₂R inhibitor PD-123,319 resulted in worsening of Ang II mediated oxidative stress as AT₂R is known to mediate the beneficial effects of Ang II stimulation.

Generation of excessive ROS leads to activation of transcription factors [nuclear factor-kappa B (NF-κB)], receptors [EGFR, prostaglandin PGE(2) (EP-1), receptor for advanced glycosylated products (RAGE)], cell signaling pathways [Janus-associated kinase-signal transducers and activators of transcription-1α (JAK-STAT), mitogen activated protein kinase (MAPK)], cytokines [interleukin-6 (IL-6), tumor necrosis factor-α (TNF-α)], serine/threonine kinases (Akt, inhibitor of nuclear factor-κB {NF-κB} kinase-β (IKK-β), ribosomal protein S6 kinase polymerase 1 (S6K1)] and other abnormal processes that disrupt the reduction-oxidation (redox) homeostasis of the cell and then the whole organ.² Specific blockade of these deleterious pathways has been shown to decrease oxidative stress-mediated effects of RAAS.² Ultimately, redox imbalances have been shown to lead to kidney injury and proteinuria. Hence, it is imperative that we understand the mechanisms through which the RAAS modulates

kidney function and pathology to better able to prevent and control deterioration of kidney function.

In this review, we will discuss RAAS modulation of kidney function, abnormal RAAS activation in kidney disease, the role of redox status in mediation of RAAS effects and some therapeutic modalities that have been shown to have salutary effects on RAAS-mediated kidney disease.

WHAT IS THE IMPORTANCE OF RAAS IN THE KIDNEY

A. Role in organ development

Among varying roles of Ang II including sodium homeostasis and regulation of aldosterone synthesis, the involvement in renal organogenesis is compelling.²¹ Angiotensinogen (Aogen) mRNA was detected by days 30–35 of gestation in the mesonephros of human embryos, while AT₁R & AT₂R were detected by day 24.²² In the human metanephros, ACE was expressed in proximal tubule cells/cortical collecting ducts (PTC/CCD), Aogen in PTC at 8 wks, AT₁R in glomeruli and PTC, and AT₂R in the undifferentiated mesenchyme.²¹ The role of Ang II in the development of kidneys is supported by evidence from deletion of the Aogen gene in mice (Agt^{-/-}). The consequent defective kidney organogenesis is lethal within 10 days of birth.^{23,24} Aogen is mainly expressed in the kidney tubules of mice by embryonic day 17 and its expression declines shortly after birth. It is known the RAAS is up-regulated during renal development and in the perinatal period; the AT₁R is expressed at high levels post partum, while AT₂R expression is higher in the kidney pre-partum.²⁵ Evidence now suggests that Ang II stimulates growth of tubular cells in vitro and induces synthesis of collagen IV mediated through the AT₁R.²⁶ Complete knockout of AT₁R results in lack of development of renal pelvis and ureteral peristaltic movement; in fact, the phenotype is mimicked by wild type mice with one ureter ligated (obstructive uropathy).²⁷ Similarly, mice with targeted mutations in the ACE, AT₁R, and in both renin genes, have nearly identical phenotypes characterized by poor survival to weaning, low blood pressure, and abnormal kidney structure. Their kidneys develop thickened with hypercellular arterial walls, interstitial fibrosis, inflammation, papillary atrophy, and tubular dilation. Interestingly, gene targeting of angiotensinogen/ACE produces a more severe phenotype when compared to AT receptor targeting. The reason could very well be the effects on non-AT receptor mediated pathways, such as aldosterone-mediated mineralocorticoid receptor (MR) activation or it could even be AT₂ receptor-mediated signaling.²⁸ As a corollary, consumption of ACE inhibition during pregnancy has been shown to result in kidney malformation mainly through defective angiogenesis/tubulogenesis. Thus, Ang II and the RAAS contribute to normal growth and development of the nephron through an interplay of AT₁R and AT₂R mediated effects and has additional roles in cell cycle progression.²⁹

B. Fluid and salt homeostasis

The classic RAAS is endocrine in function with renin secreted from juxtaglomerular apparatus (JGA) in response to low blood flows, angiotensinogen from liver and ACE from lung. Ang II is cleaved from Ang I by ACE in the blood and causes peripheral vasoconstriction and tubular reabsorption of sodium. It also increases tubular reabsorption of urinary protein (albumin). Aldosterone is secreted by adrenal glands in response to Ang II and augments Ang II mediated attempts to conserve volume by retaining sodium in the distal tubules. Ang II also has aldosterone independent effects on sodium retention via ENaC in distal tubules.³⁰ However, evidence emerging from several labs has demonstrated all components of RAAS pathway in several extra renal sites including brain, liver, lungs, heart, pancreas, kidneys, vasculature and skeletal muscle. The beneficial effects of RAAS actually become harmful when this system is

turned on excessively due to dysregulation of redox homeostasis likely due to loss of normal feedback suppression mechanisms.

RAAS IN THE KIDNEY

With the increase of newly identified components and functions, knowledge about the RAAS has significantly expanded in the last two decades. Besides “classical” RAAS, recently, compelling evidence indicates that there are also local and intracellular RAAS systems in various organs/tissues/cells. Indeed, the local RAAS has gained a great deal of attention in the pathophysiology of organ/tissue injury, particularly in the kidneys (Figure 1). It has been demonstrated that kidneys produce/express all of the RAAS components including renin, Aogen, Ang II, (pro)renin receptor, ATR's and MR.^{25,28,31–34} Local RAAS may be particularly important in kidney injury and dysfunction via induction of inflammation, cell growth, mitogenesis, apoptosis, migration, and differentiation, through activation of multiple intracellular signaling pathways (Figure 1).²¹ Although the role of circulating RAAS cannot be minimized, renal RAAS activation has been demonstrated through various paradigms to contribute to the development and progression of hypertension.^{1,35–41} Importantly, renal AT₁R activation has been shown to influence systemic hypertension compared to systemic contributions of the AT₁R. Investigators utilized a kidney cross-transplantation technique to compare four groups of mice: wild type group containing transplanted kidneys from wild type donors expressing AT_{1A} receptor (AT_{1A} in mouse is most homologous to AT₁ in humans) in the kidney transplant and in all systemic tissues, systemic AT_{1A}R KO group transplanted with kidney expressing AT_{1A}, kidney AT_{1A}R KO group expressing AT_{1A} in all tissues except for kidney and total KO group with no expression of AT_{1A}.³⁵ Blood pressure measurements between the groups suggested that systemic and kidney AT_{1A} made equal contributions to baseline blood pressure. However, when mice were stimulated with Ang II, the systemic KO group had similar pressures to wild type group suggesting that hypertension was mediated primarily through kidney AT_{1A}R and not through systemic AT_{1A}R. Additional deleterious effects of kidney AT_{1A} activation include decreased sodium excretion, weight gain, cardiac hypertrophy, atrial natriuretic peptide and brain natriuretic peptide stimulation, further demonstrating the important role of kidney AT_{1A} in cardiovascular pathology.

Renin, prorenin and (pro)renin receptor

The JGA in the kidney are known to contribute to both circulating and intra-renal renin levels. ACE inhibition increases the staining for renin by recruitment of additional cells of the afferent arterioles alongside the JGA.⁴² In addition, evidence for the expression of renin in the afferent artery, glomerular cells, proximal and distal tubular cells, possibly connecting tubule cells, exists.³¹ It is well established that the role of renin is to cleave Aogen into Ang I. Recent evidence suggests that renin also exerts direct effects via binding to (pro)renin receptor (P)RR, such as activation of extracellular signal-regulated kinases (ERK) 1/2, stimulation of transforming growth factor – β (TGF- β) and collagen I and p38MAPK.^{43,44} This receptor has been associated with circulating renin/prorenin uptake by some tissues where it may contribute to the local synthesis of Ang peptides.^{34,43,45,46} It has been reported that glomerular mesangial cells and subendothelial space of the renal vasculature specifically partition the (P)RR.⁴³ The presence of the receptor in renal tubules may contribute to tubular generation of Ang II through enhanced conversion of locally produced Aogen to Ang I.⁴⁷ In addition, a recent study indicates that prorenin plays a pivotal role in the development of diabetic nephropathy via binding to an intrinsic prorenin-binding receptor.^{48,49}

Aogen

The liver is the major organ producing Aogen. The kidneys also express Aogen, particularly in the podocytes, proximal tubules, distal convoluted tubules and renal endosomes.^{31,33,50–}

⁵³ It is also expressed weakly in the glomerular endothelial cells.¹ In addition to Aogen, the proximal tubule cells produce renin and express abundant amounts of ACE on the proximal tubule brush border. The proximal tubule cells secrete Aogen into the tubular lumen, which in turn leads to intraluminal Ang II formation with the potential to activate distal sodium retention.^{1,36} These findings indicate that Aogen can be converted into Ang II locally in the kidneys. Through elegant experiments Kobori et al showed that Aogen is not filtered across the glomerulus further demonstrating that Aogen must be locally generated from the tubules. Transgenic mice overexpressing Aogen under control of kidney androgen promoter, exhibit high expression in the proximal tubule cells and high levels of the protein in the urine once again demonstrating the existence of tubular secretion of Aogen.³⁶

ACE

ACE was believed to originate in the pulmonary epithelium, however ACE is expressed abundantly on the brush-border membrane of PTC's. In fact, it may be expressed all along the length of a nephron.⁵⁴ In contrast to rats, ACE is only minimally expressed in human vascular endothelial cells (at 10% of rat levels), which raises an important point that in humans, circulating Ang II contributes less to kidney RAAS than local RAAS itself.⁵⁵

Ang II

Intrarenal Ang II is distributed widely in the cortex and medulla.⁵⁶ Within the cortex, there is distribution of Ang II in the interstitial fluid, tubular fluid, and intracellular compartments.^{32,57,58} Studies suggest that Ang II plays an important role in proximal, distal and collecting duct sodium reabsorption.^{30,59} Intracellular Ang II has also been identified in proximal tubule cells. For example, increased Ang II was detected in renal endosomes and intramicrovillar clefts in proximal tubule cells. Ang II bound to receptors is internalized via AT₁R-mediated endocytosis.^{60–63} Internalized Ang II could be recycled and secreted to exert further actions by binding to Ang II receptors on the cell membranes. The intracellular Ang II may also bind to cytosolic receptors to stimulate the inositol 1,4,5-triphosphate pathway.⁶⁴ The intracellular Ang II may migrate to the nucleus and bind to AT₁ subtype or distinguished nuclear receptors, which in turn exert genomic actions to regulate Aogen or renin mRNA expression in proximal tubule cells.^{65,66}

MR and ENaC

Traditionally, the actions of aldosterone on the kidney have been thought to be active only in the collecting ducts. However, MR on the podocytes have been shown to mediate aldosterone mediated glomerular sclerosis.⁶⁷ Furthermore, amiloride sensitive ENaC are expressed in renal distal convoluted tubules (DCT), connecting tubules (CNT), cortical collecting ducts (CCD), and outer medullary collecting ducts (OMCD), but not in the inner medullary collecting ducts (IMCD), consistent with tighter regulation of sodium transport in the cortex and outer medulla.⁶⁸

AT₁R and AT₂R

AT₁R is expressed most prominently in the interlobular arteries and tubulointerstitial fibrous regions surrounding interlobular arteries and glomeruli, followed in decreasing order by glomeruli and cortical tubules.^{69,70} Among the tubular cells, the proximal tubule brush-border and basolateral membranes, distal tubules, cortical and medullary collecting ducts exhibited specific immunoreactivity.^{70–72} Glomerular staining for AT₁R was observed in mesangial cells and podocytes. Macula densa cells stained positively as well.⁷³ AT₂R expression has been confined to the interlobular arteries.⁷¹ The regulation of AT₁R is highly variable depending on the volume status of the animal, but in general glomerular AT₁R are decreased and tubular, increased in response to excess Ang II.¹ Upregulation of AT₁R in multiple tissues

may explain hypertension in transgenic Ren2 rats [(mRen2)27] even though plasma Ang II levels are not elevated.¹

EXCESS RAAS AND CHRONIC KIDNEY DISEASE

A. Increased intraglomerular pressure, decreased GFR, increased tubular sodium transport

Human and animal studies have shown that Ang II infusions lead to increased constriction of both afferent and efferent arterioles and lead to increases in intraglomerular pressures and decrease in GFR.¹ However, increased tubular transport of sodium may be a more significant role for Ang II in the kidney. Apical Na^+/H^+ exchange, basolateral $\text{Na}^+/\text{HCO}_3^-$ cotransport, and basolateral Na^+/K^+ -ATPase via insertion of H^+ -ATPase into the apical membrane, may be some of the mechanisms leading to increased solute retention.¹ Sustained increases in volume leads to increases in BP and the consequent long-term effects of RAAS activation. As a corollary, one would imagine that decrease in kidney tissue ACE would lead to decrease in BP. However, kidney specific ACE knockout mice did not manifest low BP and were able to maintain fluid and solute homeostasis via liver ACE alone. This shows that although kidney ACE is important, other compensatory mechanisms may come into play at the whole animal level.

B. Increased oxidative stress, altered tubuloglomerular feedback (TGF)

There is sufficient and convincing evidence to support Ang II as a key contributor to progression of kidney disease by stimulating growth, hypertrophy, oxidative stress, inflammation and fibrosis within the kidney (Figure 1). Many of the same downstream to AT_1R pathways that are invoked in the vasculature, hold true in the kidney as well (e.g. DAG/ Ca^{2+} /PKC signaling, g-protein/Rac1 signaling, MAPK/TGF- β 1 signaling, insulin growth factor-1 (IGF-1)/insulin receptor substrate-1 (IRS-1) signaling, and NAD(P)H oxidase/ROS signaling).

As described above, all the RAAS components are expressed and functional in the kidney. RAAS activation leads to activation of ROS generating enzyme systems which include metabolic oxygenases such as NAD(P)H oxidase.^{2,20,74} This enzyme complex is believed to be the major source of abnormal cellular signaling, although in terms of quantity the mitochondrial respiratory chain system may generate the most superoxide anion.^{2,74} All of the components necessary to for this enzyme complex to function in the kidney are expressed in the various kidney cell types.² Nox4 is expressed at high levels in kidney and other Nox's, including Nox1, Nox2, and Nox regulatory subunits, are expressed at lower but quantitatively significant levels making Nox enzymes attractive candidates for the origin of renal ROS including the relatively high levels of H_2O_2 seen in urine.⁷⁵⁻⁸² There appears to be no consensus for the cortico-medullary differences between the different subunits as the literature has data from different animals undergoing different treatments. Indeed, a few studies have demonstrated none or very little expression of the regulatory subunits in the medulla. In contrast, one group reported that in spontaneously hypertensive rats (SHR) the expression of Nox1, Nox2 and p67^{phox} is higher in the renal medulla when compared to the cortex and the expression of Nox4 and p22^{phox} is higher in the cortex than the medulla.⁸³ Furthermore, the expression of p47^{phox} is about the same in the cortex versus the medulla. The mesangial cells are unique in that they express Nox1, Nox4, p22^{phox} , p47^{phox} and p67^{phox} but not Nox2.^{77, 78,84,85} This may be biologically important as there is data supporting the fact that mesangial cells may be similar to vascular smooth muscle cells (VSMC's), which also lack Nox2 (expressed at very low levels). In mesangial cells, NO inhibits the expression of Nox1, suggesting a cross-talk between NO and $\text{O}_2^{\bullet-}$ generating systems.⁷⁹ p22^{phox} , p47^{phox} and p67^{phox} are all expressed in the PCT, DCT, CCD and the macula densa cells.⁸⁶⁻⁹⁰ Nox2 expression has been shown in the PCT and CCD, Nox4 in the PCT and DCT, while Nox1

expression is confined to the PCT, once again demonstrating differential regulation of redox control within the cortex. *In situ* hybridization experiments initially localized Nox4 mRNA expression to renal cortex, while the medulla showed much lower expression.^{79,81} However, immunohistochemical studies demonstrate Nox4 expression in distal portions of the human nephron, specifically, Nox4 mRNA has been demonstrated in medullary collecting ducts.^{79,81} Moreover, as mentioned above the data for Nox4 was obtained from different animal groups. Nox4 isoform of NAD(P)H oxidase is unique in that, it can also use NADH as the substrate for generating O₂^{•-}.⁹¹ This may be relevant to the kidneys, as the cortex and outer medulla preferentially use NADH as substrate and express Nox4 at high levels in these regions.⁹²

Most importantly, Nox4 is integral in the development of the renal complications of insulin resistance and hypertension.^{20,67} Increased expression of Nox4 and p22^{phox} and accumulation of 8-hydroxy-deoxyguanosine (8-OHdG), marker for ROS-induced DNA damage, in the kidney of streptozotocin-induced diabetic rats was reversed after treatment with insulin for 2 weeks.⁹³ Another group working with the same animal model showed that, infusing antisense oligonucleotides for 2 weeks targeting Nox4 mRNA was effective in reducing ROS both in cortical and glomerular homogenates, organelle hypertrophy and fibronectin expression, which are characteristic markers of diabetic kidney disease. One putative pathway proposed to increase Nox4 expression has been the Rho/Rho-kinase pathway and this was inhibited by administration of fasudil (Rho-kinase inhibitor) and statins.⁹⁴ Ang-(1–7) peptide [Ang-(1–7)] attenuated Nox4 expression, decreased NAD(P)H oxidase activity and improved proteinuria in streptozotocin treated SHR (diabetic SHR).⁹⁵ These data suggest a signaling role for Nox4-derived ROS in the development of diabetic kidney disease.⁹⁶ The Ren2 transgenic rat is a unique model for investigating the influence of kidney Ang II and the RAAS on redox control of the kidney.^{2,20,97} The Ren2 rat over-expresses the mouse renin transgene and demonstrates elevated tissue Ang II including a local renal tissue RAAS. Elevated tissue RAAS has been shown to increase oxidative stress in these animals contributing to insulin resistance, hypertension, and proteinuria. We have shown that Ang II increased Nox4 expression in the kidney/podocytes and that this was associated with proteinuria and hypertension in these animals.^{20,97} Furthermore, we have shown increased expression of NAD(P)H oxidase subunits in the glomeruli including Nox2, p47^{phox}, p67^{phox}, Rac1.^{20,97} There was increased NAD(P)H oxidase activity and increased 3-nitrotyrosine (3-NT) staining demonstrating increased ROS generation via increased enzyme activity. With the electron microscope, we have observed increased podocyte effacement, basement membrane thickening, slit pore diaphragm widening and decrease in slit pore numbers in Ren2 transgenic rat and these defects were ameliorated with ARBs, superoxide dismutase mimetic (tempol).^{20,97} We have also observed that blockade of both RAAS and ROS ameliorate proteinuria thereby implicating a RAAS-mediated redox imbalance pathway.²⁰ Blockade of RAAS/ROS also abrogated changes of nephrosclerosis at the structural level. Importantly, these experiments revealed that modulation of the RAAS, independent of blood pressure, had salutary effects on kidney function.

Several groups have demonstrated that ROS can decrease both NO generation and function in the kidney.¹⁹ ROS has been shown to convert NO to a ROS species called peroxynitrite (ONOO⁻). NO decreases TGF thereby exquisitely controlling retention of sodium till the body extracellular fluid is replete. Peroxynitrite acting in the JGA can increase the tubuloglomerular feedback leading to retention of sodium by the tubules even though the body is replete with sodium, resulting in hypertension and volume overload. Importantly, blockade of AT₁R ameliorated ROS induced increases in TGF, linking RAAS activation to ROS-mediated redox alterations.¹⁹

C. Clinical studies

RAAS-mediated mechanisms for ROS production in the kidney are currently a subject of intense interest in the nephrology community. This is mostly due to K-DOQI identifying this area as a focus of research and the existence of identifiable human disease from over-expression/mutations/polymorphisms in the renin, Aogen, ACE, AT₁R, aldosterone, and MR genes.² An example of this principle exists in a Dutch cohort with insulin-dependent diabetes mellitus (IDDM)-induced incipient diabetic kidney disease.⁹⁸ Within this population, the T-allele of the Aogen-M235T polymorphism is associated with increases in proteinuria and the CC-genotype of the AT₁-A1166C polymorphism is associated with hypertension. In addition, mutations in other components of RAAS pathways are also associated with hypertension. However, monogenetic forms of HTN constitute only 5% of total disease burden and the search for better markers of disease e.g. defining phenotypes by clustering of factors such as hyperinsulinemia, cholesterol and lipids, waist circumference and hyperglycemia is gaining momentum. Importantly, the debate about identifying renal disease by markers other than proteinuria has intensified urging investigators to look for alternate and more robust markers of CKD. 8-OHdG is one such marker that can be measured in the urinary samples of patients and indicates oxidative stress and damage to glomerular filtration barrier injury. Recently, better assays (ELISA) have been developed to measure Aogen in the urine and plasma, these remain to be tested in humans and on a larger scale.⁹⁹

THERAPY FOR EXCESS RAAS-MEDIATED KIDNEY INJURY

a. ARBs/ACEi's (Valsartan, Irbesartan, losartan,)

Several well designed clinical trials have guided us in therapy of elevated RAAS in hypertension and diabetic kidney disease. These include the clinical trials listed in Table 1. The mechanism for improvement in glomerular filtration barrier injury and/or proteinuria has been proposed to be BP dependent or BP independent. The BP independent mechanisms for benefit from RAAS blockade include mitigation of pro-inflammatory and pro-fibrotic pathways (Figure 1). We and others have used transgenic rat models and genetic models of hypertension and glomerular filtration barrier injury to demonstrate benefit from RAAS blockade with ARBs/ACE inhibitors including valsartan, irbesartan and losartan. We have demonstrated that while BP was reduced with these drugs, the tissue benefits were beyond that anticipated by BP control alone including amelioration of proteinuria, slit diaphragm widening, podocyte effacement, decrease in number of slit pores and basement membrane widening. We have also shown decrease in perivascular fibrosis with Verhoeff-van Gieson (VVG) stain and trichrome; decrease in oxidative stress with 3-NT in glomeruli, 8-OHdG in urine, NAD(P)H oxidase activity, enzyme subunits by western blots and immunohistochemistry; translocation of Rac1 to plasma membrane, decreased transcription of subunits. Others have demonstrated that ARB's decrease glomerulosclerosis in Streptozotocin-diabetic rats, SHR and Zucker obese rats.² Telmisartan an ARB with unique peroxisome proliferator activator receptor- gamma (PPAR- γ) agonist activity may have effects on insulin sensitivity in addition to its blood pressure lowering effects sans the nagging side effects of full PPAR- γ agonists e.g. fluid retention (thiazolidinediones).

b. Control of oxidative stress-antioxidants (tempol, apocyanin, DPI)

That there are BP independent effects of RAAS has been made prominent initially through ROS blockade. Although there may be some debate about whether it is NAD(P)H oxidase or other oxygenases, it is clear that reduction in NAD(P)H oxidase activity and hence ROS, can ameliorate glomerular filtration barrier injury on its own. However, the full spectrum of benefit that stems from ARBs/ACE inhibitors is not evident. Other novel ways to block NAD(P)H oxidase activity including gp91 ds-tat, siRNA, monoclonal antibodies are being tried and hold

great promise for not only hypertension but also for other disease processes with elevated oxidative stress.

c. Statins (rosuvastatin)

These drugs have great potential in the blockade of RAAS-mediated kidney injury through reduction of oxidative stress via inhibition of Rac1 translocation to the membrane, possible reduction in inflammation via NF- κ B, IL-6, C-reactive peptide (hsCRP) and TNF- α .^{97,100} Again, the benefits are BP-independent, through decrease in inflammation and fibrosis, increase in plaque stability and, finally, decrease in glomerular filtration barrier injury.

d. MR antagonists (spironolactone)

Spironolactone was originally used as an antihypertensive due to its aldosterone blocking effects in the distal tubules, however it is well known now over the past 5–7 years that aldosterone has roles similar to Ang II in tissue inflammation and fibrosis. RALES and EPHESUS have shown that addition of spironolactone to New York Heart Association (NYHA) 2/3 and post myocardial infarction (MI) patients, improves morbidity and mortality significantly.² Our lab has shown that this drug can ameliorate oxidative stress, improve insulin sensitivity/resistance in the skeletal muscle and kidney (manuscripts submitted). Interestingly, the benefits were evident without major reductions in BP. MR receptors have been demonstrated in the kidney by Shibata et al (and unpublished work) and eplerenone was used to demonstrate improvement in glomerulosclerosis in Sprague Dawley rats undergoing aldosterone infusions.⁶⁷

e. Renin blocker (Aliskiren)

Direct renin inhibition has been shown through recent clinical trials to have added benefits to baseline ARBs/ACE inhibitors further demonstrating the usefulness of RAAS blockade (AVOID trial).¹⁰² Aliskiren, the first orally available drug in its class, blocks renin and prorenin by binding to their active site. It inactivates prorenin by keeping it in the open configuration. In addition, it has also been postulated to bind to (P)RR, decrease the gene expression of (P)RR and alter the 3D configuration of renin.¹⁰¹ However, it may not alter the binding of renin to (P)RR.¹⁰⁰ We and others have shown that independent of BP reduction, direct renin inhibition leads to reduction in 3-NT staining, decrease in perivascular fibrosis, albuminuria, improved insulin sensitivity in ex vivo samples (unpublished observations).¹⁰¹

f. β -blockers (Nebivolol?)

The search for drugs that can be combined for maximum benefit sometimes leads to drugs that have dual effects. One such drug is the β -blocker Nebivolol that may possess vasodilatory properties in addition to traditional selective β -blockade. Nebivolol has been proposed to achieve these effects via recruitment of endothelial nitric oxide synthase (eNOS) and decrease in superoxide activity.¹⁰³ This has sparked off interest in the use of this drug in preventing complications from CVD and CKD.

FUTURE PERSPECTIVES

The role of local RAAS in normal function of the kidney and pathogenesis of chronic kidney disease is complex. The RAAS interacts with a myriad of cytosolic factors, cellular and extracellular signaling pathways for seamless control of cellular mechanisms and the kidney as a whole. Dysregulation of these intracellular processes contribute to an imbalance in redox homeostasis and oxidative stress. Our understanding of renal NAD(P)H oxidase is expanding in many of the RAAS-mediated effects, which lead to increased ROS formation, oxidative stress, redox imbalance and hypertension.

Small animal and genetic investigations have expanded our understanding of hypertension and chronic kidney disease. The incorporation of various cell lines have further contributed towards understanding basic signaling pathways. However, our understanding of signaling pathways among the numerous cells within the kidney and how they contribute to renal function is variable at best. Modification of the RAAS mediated oxidative stress in the kidney has provided our best clinical understanding of renal redox handling as it relates to kidney function and hypertension.

Identifying chronic kidney disease early and modifying the risk factors has become a prerogative of many nations that are faced with the epidemic of this disease. Although proteinuria has been used as marker in many clinical trials, its correlation with hard renal outcomes is variable at best. Understanding the interplay of RAAS with redox mechanisms in chronic kidney disease may provide new markers to test for reliably identifying CKD early in its course. Further development of podocyte biology will likely facilitate this process.

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Figure 1.

This figure illustrates the renin-angiotensin-aldosterone system (RAAS) in and around a cell in the kidney. Post receptor signaling is a complicated process and has been minimized to emphasize the link between RAAS and oxidative stress, inflammation, insulin resistance and sclerosis. Ultimately, the result of an overactive kidney RAAS is associated with hypertension and GFB injury. Sites of action for RAAS blocking drugs are depicted. ACE = angiotensin converting enzyme, ARB = angiotensin receptor blocker, MR = mineralocorticoid receptor, GFB = glomerular filtration barrier

Table 1
Clinical trials showing renal benefits of RAAS blockade in Kidney Function

Category of DKD/DN	Trial	Patient Population	N	Treatment Arms	Primary End Point	Final Outcome
I/II	HOPE ^{5,6}	High-risk patients with and without known Diabetes	9297	Ramipril/Placebo	Overt proteinuria	Ramipril reduced risk of any progression (OR, 0.87; 95% CI, 0.78 to 0.97; $P = 0.0146$).
III	IRMA 2 ⁷	Type 2 Diabetes with MA	590	Irbesartan 150mg or 300mg/Placebo	Time to onset of Diabetic Nephropathy	Irbesartan reduced risk of primary end point (HR 0.30; 95% CI: 0.14–0.61; $P < 0.001$ for 300 mg irbesartan vs placebo); (HR 0.61; 95% CI: 0.34–1.08; $P < 0.08$ for 150 mg irbesartan vs placebo)
II/III	SOLVD ^{8,9}	Diabetic & non-diabetic with microalbuminuria	5590	Enalapril vs placebo	Time to clinical proteinuria	AER decreased by 16.7 in enalapril group and prevented clinical proteinuria in diabetic patients (0.38 [0.17–0.81], $P = 0.0123$)
III	MARVAL ¹⁰	Type 2 Diabetes with MA	332	Valsartan/Amlodipine	% change in UAER from baseline to 24 wks.	UAER at 24 wks was reduced by 44% with valsartan and 8% with amlodipine ($P < 0.001$); valsartan significantly reversed MA to normal albuminuria
III	INNOVATION ¹¹	Type 2 Diabetes and UACR 100–300mg/g, Cr < 1.5mg/dl (men) and < 1.3mg/dl (women).	527	Telmisartan (80/40 mg)/Placebo	Transition rate from Incipient to Overt Nephropathy, UACR>300 mg/g, and increase $\geq 30\%$ from baseline at 2 consecutive 4 week visits.	Transition rates to Overt Nephropathy were 16.7% with 80 mg Telmisartan ($n = 168$), 22.6 % with 40 mg

Category of DKD/DN	Trial	Patient Population	N	Treatment Arms	Primary End Point	Final Outcome
III	BENEDICT ¹²	Type 2 Diabetes and HTN but with normoalbuminuria	1204	Tandolapril/Verapamil/Placebo.	Development of persistent MA	Telmisartan (<i>n</i> = 172), and 49.9% with placebo (<i>n</i> = 174) (Both telmisartan doses vs placebo, <i>P</i> < 0.0001). For Developing End Point AF = 0.39 for Tandolapril + Verapamil v/s Placebo. AF = 0.44 For Tandolapril V/s Placebo and AF = 0.83 with Verapamil v/s placebo.
IV	IDNT ¹³	Hypertension with Diabetic Nephropathy	1715	Irbesartan 300mg/Amlodipine 10mg/Placebo	Composite of development of ESRD, doubling Cr. or death	Irbesartan significantly reduced primary composite end point by 20% compared to placebo (<i>P</i> < 0.02) and by 23% compared to amlodipine (<i>P</i> < 0.006).
IV	RENAAL ¹⁴	Type 2 Diabetes with Nephropathy	1513	Losartan/Placebo	Composite of doubling of Cr. development of ESRD.	Losartan significantly reduced the composite end point by 16% (<i>P</i> < 0.02), but not death.

BL indicates baseline; CI, confidence interval; RR Relative Risk; HR, Hazard Ratio; Cr, serum creatinine; CV, cardiovascular; HF, Heart Failure; CAD, Coronary Artery Disease, MA, microalbuminuria; ESRD, End Stage Renal Disease; RPF, Renal Plasma Flow; MI, myocardial infarction; N, number of patients enrolled; UAER, urine albumin excretion; wk, weeks; GFR, Glomerular Filtration Rate; NIDDM, noninsulin-dependent diabetes mellitus; HOPE, Heart Outcomes Prevention Evaluation study; IRMA, Irbesartan in patients with type 2 diabetes with MicroAlbuminuria study; IDNT, Irbesartan Diabetic Nephropathy Trial; MARVAL, MicroAlbuminuria Reduction with VALsartan trial; RENAAAL, Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist, Losartan; BENEDICT, Bergamo Nephrologic Diabetes Complications Trial; INNOVATION, IncipieNt to OVert: Angiotensin II receptor blocker, Telmisartan, Investigation On type 2 diabetic Nephropathy