Oxidative Stress in Hypertension and Chronic Kidney Disease: Role of Angiotensin II

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Angiotensin II, via the type 1 (AT1) receptor, stimulates oxidative stress. The vasculature, interstitium, juxtaglomerular apparatus, and the distal nephron in the kidney express nicotinamide adenine dinucleotide phosphate (NADPH) oxidase that generates superoxide anion, which is an important component of angiotensin II-induced oxidative stress. The angiotensinogen gene is stimulated by NF-kappaB activation, which is sensitive to the redox ratio, providing a positive feedback loop that can upregulate angiotensin II production. Oxidative stress can accompany hypertension in many models, including the spontaneously hypertensive rat (SHR), angiotensin II-infused rats, renovascular hypertension, and the deoxycorticosterone acetate (DOCA) salt model of hypertension. AT1 receptor antagonists can abrogate the effects of angiotensin II on oxidative stress, thus providing an important mechanistic insight onto the renal protective effects of these agents in conditions associated with angiotensin II excess.
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REACTIVE OXYGEN SPECIES, especially superoxide and hydrogen peroxide, are important signaling molecules. Their production is regulated by enzymes such as the vascular nicotinamide adenine dinucleotide phosphate (NADPH) oxidases, and their catabolism by antioxidant enzymes such as superoxide dismutase, catalase, and glutathione peroxidase.[1] Both superoxide and hydrogen peroxide serve as second messengers to activate multiple intracellular proteins and enzymes that in turn activate redox-sensitive transcription factors. Thus, reactive oxygen species participate in vascular smooth muscle cell growth and migration; modulation of endothelial function, including endothelium-dependent relaxation and expression of adhesion molecules, chemoattractant compounds and cytokines rendering a proinflammatory phenotype; and modification of the extracellular matrix.

The urinary environment is a pro-oxidant one with measured amounts of hydrogen peroxide attaining micromolar quantities in the urine of rats and humans.[2,3] The kidney, therefore, is particularly susceptible to oxidative stress. Oxidative stress in the kidney can contribute to progressive renal disease by virtue of renal hemodynamic actions, by altering glomerular permeability, by inducing cellular growth and apoptosis, and by promoting acute and chronic inflammatory responses.[4-8]

Angiotensin II stimulates oxidative stress.[9,10] The vasculature, interstitium, juxtaglomerular apparatus, and the distal nephron in the kidney have a rich expression of NADPH oxidase that generates superoxide anion, which is important in transducing the signal of angiotensin II to oxidative stress. Besides the direct effect of free radicals, they can also quench nitric oxide, an endothelium-dependent vascular relaxant, and thereby aggravate angiotensin II-induced vasoconstriction. The angiotensinogen gene, which provides the precursor for angiotensin production, is stimulated by NF-kappaB activation, which is sensitive to the redox ratio. This provides a positive feedback loop that upregulates angiotensin II production.[6]

Diverse vasoconstrictor mechanisms, including blockade of nitric oxide synthase, and activation of angiotensin II type 1 (AT1) receptors and thromboxane receptors, can induce oxidative stress in hypertension.[11] The effects of superoxide anion and hydrogen peroxide on vascular smooth muscle cells can cause vasoconstriction by quenching of nitric oxide and by nitric oxide synthase-independent mechanisms that include increased generation of endothelin-1. Oxidative stress can accompany hypertension in many models, including the spontaneously hypertensive rat (SHR), angiotensin II-infused rats, renovascular hypertension, the deoxycorticosterone acetate (DOCA) salt model, and obesity-related hypertension.

Several approaches have been used to study the link between angiotensin II, induction of oxidative stress and the consequences of oxidative stress at

Table 1. Summary of Laboratory Methods for Measuring Oxidative Stress

Method	Significance	Rationale	Interpretation
Thiobarbituric acid reactive substances (TBARS)	The formation of lipid hydroperoxides by oxidative lipid damage leads to dysfunction of membrane-bound receptors, and these compounds possess cytotoxic and mutagenic properties, which are thought to play a major role in aging and atherosclerosis	Peroxidation of polyunsaturated fatty acids are derivatized with thiobarbituric acid to yield a red compound that is measured colorimetrically	Increase in TBARS is indicative of lipid peroxidation; the test is simple, however, it has low sensitivity and low specificity as a result of interfering chromogens
Malondialdehyde (MDA)	As above	Malondialdehyde is a byproduct of lipid peroxidation and is formed by β-scission of peroxidized polyunsaturated fatty acids	Measurement by HPLC or other noncolorimetric methods (eg, mass spectroscopy) are more sensitive and specific compared with TBARS
C11-Bodipy 581/591	In vivo marker of lipid peroxidation	Redox-sensitive fluorescent probe that changes color with generation of lipid-derived free radicals	
Protein carbonyl content	Protein dysfunction occurs with carbonylation	Oxidative damage to proteins lead to insertion of carbonyl groups in certain aminoacids; carbonyl groups can be detected by derivatizing with dinitrophenyl hydrazine, separating the derivatizing reagent from the proteins (washing protein pellet or size exclusion chromatography) and measuring the absorbance at 360 nm; ELISA method to detect dinitophenyl hydrazone is also available	Washing the protein pellet, size exclusion chromatography, ELISA have all been used to estimate protein carbonyl content; albumin appears to be particularly susceptible to carbonylation
Nitrotyrosine	Membrane dysfunction by nitrosylation of lipid hydroperoxides or tissue protein (tyrosine residues) could be important in atherogenesis, vascular dysfunction, and oxidative stress	The reaction of nitric oxide with superoxide anion yields peroxynitrite that can cause nitrosylation of tyrosine residues of proteins; however, nitrotyrosine is not a specific footprint of peroxynitrite reaction in biology; the oxidation of nitrite by myeloperoxidase-derived hypochlorous acid yields nitryl chloride, which can also form 3-nitrotyrosine	Increased levels suggest nitrosylation stress; HPLC or gas chromatography are available; in tissues or cells, immunochemical detection of nitrated proteins in typically used
8-hydroxy-2'-deoxyguanosine	Oxidative DNA damage by free radicals produces 8-hydroxy-2'-deoxyguanosine		Urinary excretion of this compound is a marker of DNA damage
Heme oxygenase 1 activity	A redox-sensitive enzyme that converts heme to biliverdin and in the process produces carbon monoxide and releases iron; enzyme is activated in states of oxidative stress, by heme and cytokines; hepatic and renal damage is seen in HO-1 knockout mice	Effect of activators (hemin) and inhibitors (zinc protoporphyrin) or alternatively, overexpression or knockout by genetic methods can be used to study the effects of modulation of the enzyme activity	Increased enzyme activity reduces oxidative stress by the disposal of oxygen free radicals
8-iso Prostaglandin $F_{2\alpha}$	Prostaglandin metabolite with vasoconstrictor and sodium-retaining effects on the kidney	An isoprostane produced as a result of oxidative stress	Increased levels associated with oxidative stress; quantitation of F2 isoprostanes requires mass spectroscopy methods for most reliable detection, however, most investigators have used immunoassays

Table 1. Summary of Laboratory Methods for Measuring Oxidative Stress (Cont'd)

Method	Significance	Rationale	Interpretation
Superoxide dismutase		Enzyme responsible for the neutralization of the superoxide anion, a highly reactive free radical, to hydrogen peroxide and oxygen; the Cu, Zn superoxide dismutase comprises 90% of the total SOD activity	Reduced blood pressure or vasorelaxation with SOD mimetic tempol is indicative of oxidative stress.
Tempol infusion		Mimics the activity of superoxide dismutase; thus, there is increased disposal of superoxide with tempol infusion	Reduced blood pressure or vasorelaxation is indicative of superoxide anion-mediated effects
Catalase		An antioxidant enzyme responsible for catalyzing the removal of hydrogen peroxide	Reduced activity indicates reduced ability to quench hydrogen peroxide
Glutathione peroxidase		An antioxidant enzyme responsible for generating reduced glutathione	Reduced activity indicates reduced ability to generate glutathione
Xanthine oxidase		Normally present in endothelial cells, catalyzes the degradation of hypoxanthine to uric acid; superoxide, hydrogen peroxide, and the hydroxyl radical are produced as byproducts	Reduced blood pressure or vasorelaxation with inhibitor such as oxypurinol or allopurinol is indicative of oxidative stress
Superoxide anion production	In the blood vessels production is related to NADPH oxidase activity that is sensitive to angiotensin II effect	A highly active free radical, mediates intracellular signaling, gene activation, inflammation, vasoconstriction	Chemiluminescent probes such as luminol, lucigenin, and coelenterazine are commonly used for the detection of oxygen radical intermediates in biologic systems
Reduced to oxidized glutathione ratio	Reduced glutathione is an antioxidant that is oxidized to its disulfide; the ratio of the oxidized to total glutathione can be used to identify oxidative stress	Only a small amount of glutathione is present in the oxidized form, thus, prevention of in vitro oxidation could yield a falsely low ratio, trapping reduced glutathione with N-ethylmaleimide before derivatization with dinitrofluorobenzene and subsequent analysis by HPLC or capillary electrophoresis	High oxidized to total glutathione is indicative of oxidative stress

Abbreviations: ELISA, enzyme-linked immunosorbent assay; HPLC, high-pressure liquid chromatography; SOD, superoxide dismutase; NADPH, nicotinamde adenine dinucleotide phosphate.

the tissue level. Animal models and in vitro cell culture studies are discussed followed by human studies that implicate the role of angiotensin II in causing oxidative stress. This review discusses the role of angiotensin II, specifically by activation of AT1 receptors, in the pathogenesis and consequences of oxidative stress in chronic kidney disease.

WHOLE ANIMAL STUDIES OF THE EFFECTS OF ANGIOTENSIN II

Two general approaches have been used to investigate the role of angiotensin II in causing oxidative stress. Infusion models, in which angiotensin II is administered by minipumps, have been used to examine the effects of angiotensin II on oxidative stress and tissue injury. For the sake of convenience, knockout and transgenic models are considered in this category. In the second model, renal injury has been inflicted without infusing angiotensin II, but the association of angiotensin II with oxidative stress has been implicated by administration of AT1 receptor antagonists.

Methodology used to assess oxidative stress is summarized in Table 1.

Angiotensin II Infusion Models

Haugen et al[12] examined the capacity of angiotensin II to induce oxidative stress in vivo and the functional significance of such stress. Three models were used to assess the direct effect of angiotensin II. In the first model, angiotensin II was administered by miniosmotic pumps to rats maintained on standard diets. In the second model, rats were made hypertensive with DOCA and salt, which would be expected to suppress the renin–angiotensin system. In the third model, rats maintained on antioxidant-deficient diets were studied. Angiotensin II administered in vivo increased kidney content of thiobarbituric acid reactive substances (TBARS) and protein carbonyl content in the first models. In addition, the oxidant-sensitive gene, heme oxygenase-1 (HO-1), activity was also increased as a result of induction of HO-1 mRNA in the proximal tubule, whereas constitutive HO-2 mRNA was not affected by angiotensin II infusion. The renal oxidative stress indices were not increased in rats treated with DOCA and salt for 3 weeks. The increase in oxidative stress indices observed with angiotensin II infusion appeared to be functionally significant, because administration of angiotensin II to rats maintained on a prooxidant diet was associated with increased proteinuria and decreased creatinine clearance more than that seen with a standard diet. Taken together, these experiments offer direct evidence that angiotensin II induces oxidative stress in vivo, especially in the renal proximal tubules, and volume expansion and induction of HO-1 activity appear to minimize the deleterious effects of increased oxidative stress.[12]

Kawada et al[13] studied the relationship between prolonged infusions of angiotensin II and delayed rise in blood pressure (BP). Mice were subcutaneously infused with 200, 400, and 1000 ng/kg/min of angiotensin II. The systolic BP increased by day 3 at the highest dose but showed a delayed rise (days 9–13) at the lower infusion rates. At day 6, renal hemodynamic changes were seen at the lower dose without concomitant changes in systemic BP. The mean arterial pressure at 400 ng/kg/min angiotensin II was not elevated, yet the glomerular filtration rate (GFR) and filtration fraction were increased, consistent with increased postglomerular vascular resistance. From day 6 through day 14, the mean arterial pressure increased and was accompanied by a significant reduction in GFR and

elevation of renal vascular resistance, consistent with increased preglomerular vascular resistance. Renal excretion of 8-iso prostaglandin $F_{2\alpha}$ was increased 2.3-fold at day 12, indicating increased oxidative stress in response to the angiotensin II infusion. Concurrent administration of tempol, a superoxide dismutase (SOD) mimetic reduced the effects on BP, renal vascular resistance, and renal excretion of 8-iso prostaglandin $F_{2\alpha}$ at 2 weeks. Thus, increased oxidative stress was implicated in the increase in the BP and renal vascular resistance in this model of chronic angiotensin II infusion in the mouse.[13]

Nishiyama et al.[14] have demonstrated that the hypertensive response of angiotensin II is partly the result of inactivation of nitric oxide through the generation of oxygen-derived free radicals. Angiotensin II-infused rats became hypertensive and increased vascular superoxide production twofold. This effect was normalized by treatment with tempol, which significantly reduced mean arterial pressure and systemic vascular resistance in angiotensin II-infused rats but had no effect on blood pressure in vehicle-infused rats. Infusion of a nitric oxide synthase inhibitor markedly attenuated the systemic and regional hemodynamic responses of tempol in angiotensin II-infused rats, indicating that quenching of nitric oxide by angiotensin II-induced free radical generation contributes to the hypertensive response.[14]

Angiotensin II has important cardiovascular effects through the generation of oxidative stress. Oxidant stress increases vascular endothelial permeability and promotes leukocyte adhesion, which are coupled with alterations in endothelial signal transduction and redox-regulated transcription factors such as activator protein-1 and NF-kappaB. Rats receiving angiotensin II for 10 days manifest AT1-mediated hypertension and LOX-1 upregulation, a novel endothelial receptor for oxidized low-density lipoprotein, in aortic endothelium.[15] Tempol, a SOD mimetic, alleviated LOX-1 augmentation induced by angiotensin II, implicating the role of oxidative stress in atherosclerosis. Hemodynamic function, oxidative stress, and left ventricular mass were all improved with AT1 receptor blockade in a rat model of myocardial infarction and congestive heart failure; reactive oxygen species generation in neutrophils and aortic rings, and lipid peroxidation have been noted with angiotensin II infusions.[16]

In angiotensin II-infused rats,[17] NADPH oxidase activity and vascular wall inflammation were modulated by a peroxisome proliferator-activated receptor-alpha activator. In addition, the development of hypertension was attenuated, structural abnormalities were corrected, and endothelial dysfunction was improved, all consistent with decreased oxidative stress and inflammation in the vascular wall.

The role of aldosterone, as a mediator of angiotensin II-induced vascular, structural, and functional alterations, was examined in Sprague-Dawley rats.[18] Angiotensin II and aldosterone-induced increases in systolic BP was reduced by spironolactone. Aortic NADPH oxidase activity was increased by angiotensin II and aldosterone and was reduced by spironolactone. Plasma TBARS (a marker of oxidative stress) was higher in angiotensin II- and aldosterone-treated rats and was normalized by spironolactone. These findings suggest that aldosterone could mediate some of angiotensin II-induced vascular effects in hypertension that are related to increased oxidative stress.

The role of angiotensin II AT1 versus type 2 receptors (AT2-R) was examined in the oxidative stress that follows angiotensin II infusion.[19] Angiotensin II was infused subcutaneously in rats for 1 week, and excretion of 8-iso prostaglandin $F_{2\alpha}$ and malonyldialdehyde (MDA) were related to renal cortical mRNA abundance for subunits of NADPH oxidase and superoxide dismutases using real-time polymerase chain reaction. Subsets of infused rats were given candesartan cilexetil or the AT2 receptor antagonist PD-123,319. Compared with vehicle, angiotensin II increased 8-iso prostaglandin $F_{2\alpha}$ and MDA excretion by 41%. This was prevented by candesartan and increased by PD-123,319. Compared with vehicle, angiotensin II doubled renal cortical mRNA expression of p22phox (NADPH oxidase subunit) and Nox-1 (NADPH oxidase isoform), and decreased expression of Nox-4 and extracellular SOD. Candesartan prevented all of these changes, whereas PD-123, 319 accentuated changes in p22phox, Nox-1, and p67phox. These results demonstrated that in the rat angiotensin II infusion model, oxidative stress is stimulated by the AT1 receptors, which is countered by protective effects of the AT2 receptors[19]

Angiotensin II Effects in Transgenic Models

The role of angiotensin II in causing oxidative stress and vascular dysfunction was studied by Mervaala and colleagues in rats that are double-transgenic for human renin and human angiotensinogen genes and develop angiotensin II-mediated hypertension.[20] In 7-week-old hypertensive rats, the endothelium-mediated relaxation was markedly impaired. Preincubation with SOD or oxypurinol (xanthine oxidase inhibitor) improved endothelium-dependent vascular relaxation, indicating a role of increased free radical formation in causing endothelial dysfunction in this model. Markers of total body nitric oxide generation were decreased by 85%, serum 8-iso prostaglandin $F_{2\alpha}$ was increased by 100%, and the activity of xanthine oxidase/reductase in the kidney was increased by 40%. Valsartan, an AT1 receptor antagonist, normalized BP, endothelial dysfunction, serum 8-iso prostaglandin $F_{2\alpha}$ levels, renal xanthine oxidase/reductase activity, and nitric oxide generation. These studies demonstrate that role of AT1 receptor-mediated endothelial dysfunction and its association with increased oxidative stress and vascular xanthine oxidase activity.[20]

Taking the opposite approach as the above investigators, Wang et al.[21] studied knockout mice that are genetically deficient in gp91phox, an NADPH oxidase subunit protein. The baseline BP was significantly lower (92 mm Hg) in these mice than in wild-type (101 mm Hg), but infusion of angiotensin II for 6 days caused similar increases in BP in both groups. Whereas angiotensin II increased aortic superoxide anion production two-fold in the aorta of wild-type mice, there was no such effect in the knockout mice. Aortic medial area was not increased in knockout animals. Levels of reactive oxidant species (3-nitrotyrosine immunoreactivity) were increased in those regions expressing NADPH oxidase in wild-type but not in knockout mouse aortas in response to angiotensin II. These results indicate an essential in vivo role for NADPH oxidase-derived superoxide anion in the regulation of basal BP, and in vascular hypertrophic and oxidant stress responses to angiotensin II that can be shown independently of changes in blood pressure.[21]

Role of Dietary Salt Intake

Dietary sodium intake can modulate oxidative stress and target organ damage induced by angiotensin II. Rugale et al.[22] examined the influence of a low-sodium diet after a 10-day infusion of angiotensin II (200 or 400 ng/kg/min). Although the final tail-cuff pressure was similar in low sodium

and normal sodium rats infused with either dose of angiotensin II, the increase in heart weight index on high-dose angiotensin II was prevented by the low-sodium diet. Sodium restriction reduced the rise in albuminuria, and the increased production of superoxide anion and hydrogen peroxide was abrogated by the low-sodium diet, even though the hypertensive response to angiotensin II infusion was the same as in the rats receiving the usual sodium diet.[22] Cheng et al.[23] found that increased dietary sodium intake dramatically increased 8-iso prostaglandin $F_{2\alpha}$ production in a spontaneously diabetic rat model. On the other hand, hypertension induced by DOCA and salt in the rat, an angiotensin II-independent model of hypertension appears to protect from oxidative stress.[12] These studies suggest that excess dietary salt can acutely result in a pro-oxidant state, and that the degree of associated tissue damage is more directly related to the severity of the oxidative stress rather than the absolute increases in the systemic BP.

Renal Disease Models Associated With Increased Angiotensin II Production

Angiotensin II can serve as an important determinant of progressive renal injury in models of renal disease. The use of angiotensin II antagonists in these models can provide important information on the role of angiotensin II in causing oxidative stress and the impact of that effect on the tissue damage.

Lerman et al.[24] measured markers of oxidative stress and renal blood flow in pigs after induction of unilateral renal artery stenosis compared with a sham operation. Five weeks after the procedure, plasma renin activity and mean arterial pressure were elevated and the pigs became hypertensive. Levels of 8-iso prostaglandin $F_{2\alpha}$ were significantly increased, indicating that oxidative stress correlated with both plasma renin activity and arterial pressure. By 10 weeks, plasma renin activity returned to baseline but arterial pressure remained elevated. Nevertheless, 8-iso prostaglandin $F_{2\alpha}$ levels remained elevated and still correlated directly with the increase in arterial pressure. Therefore, early renovascular hypertension is associated with an increase in plasma renin activity, arterial pressure, and increased systemic oxidative stress. Oxidative stress, as indicated by increased 8-iso prostaglandin $F_{2\alpha}$ production, could be sustained along with the hypertension despite lowering of plasma renin activity, presumably reflecting secondary volume expansion that occurs in this model. Even though the plasma renin activity is suppressed, the ongoing oxidative stress could contribute to progressive target organ damage.

The SHR exhibits angiotensin II-dependent oxidative stress and reduced efficiency of renal oxygen use indexed for tubular sodium transport. Welch et al.[25] investigated the hypothesis that angiotensin II mediates the inefficient renal oxygenation in the SHR that is presumably associated with free radical production. Groups of SHR and Wistar Kyoto (WKY) rats received vehicle, candesartan, or triple antihypertensive therapy (hydralazine, hydrochlorothiazide, and reserpine) for 2 weeks. Renal oxygen use efficiency measured from cortical pO_2 in the kidney indexed for tubular sodium transport was reduced in the SHR kidney compared with the WKY. This difference was corrected by AT1 receptor blockade and was largely independent of changes in BP. Similar results have been demonstrated in the clipped kidney of the early two-kidney, one-clip angiotensin II-dependent model of hypertension in the rat.[26] Other studies by this group[27] demonstrated that exaggerated tubuloglomerular feedback was associated with hypertension in the SHR rat. AT1 receptor blockade diminished oxidative stress and restored nitric oxide signaling in the juxtaglomerular apparatus of the SHR, implicating intrarenal angiotensin II effects on tubuloglomerular feedback.

Immunocompetent cells infiltrate the kidney in several models of experimental hypertension.[28] Reduction of this infiltrate results in prevention of salt-sensitive hypertension induced by short-term angiotensin II infusion and nitric oxide inhibition.[29,30] In the SHR rat, BP was reduced to normal levels by treatment with mycophenolate mofetil (MMF) in association with a reduction in lymphocyte, macrophage, and angiotensin II-positive cells infiltrating the kidney. Oxidative stress was also reduced by MMF, as indicated by a reduction in urinary MDA, renal MDA content, and superoxide-positive cells, and was highly correlated with BP levels. Renal infiltration with immune cells plays a major role in the hypertension in SHR,[28] and other forms of salt-sensitive hypertension,[31] and could be responsible for the angiotensin II-mediated increase in oxidative stress and tissue injury in this model.

Bapat et al.[32] reported that chronic nitric oxide synthase inhibition leads to renal injury, hypertension, and proteinuria in a rat model. They studied

in vivo lipid peroxidation with the reactive oxygen sensitive probe, C11-Bodipy 581/591. In this model, activation of the stress-induced cytoprotective protein, HSP70, preceded any oxidative damage. The increase in oxidation was dependent on angiotensin II and NADPH oxidase and prevented by vitamin E. Interestingly, in this model of chronic nitric oxide synthase inhibition, the damage by oxidative stress was seen predominantly in tubules, and not in glomeruli or blood vessels as might be expected if a hemodynamic response to angiotensin II was the primary cause of injury. Kitamoto et al.[33] demonstrated benefits of AT1 receptor blockade on superoxide anion production and oxidative stress parameters in the aortas of animals treated chronically with nitric oxide synthase inhibitors.

Chade et al.[34] studied renal injury in hypercholestrolemic pigs. Compared with control animals, the hypercholestrolemic pigs had blunted renal cortical perfusion and augmented tubular response to acetylcholine that were restored to control levels with AT1 receptor blockade with irbesartan. Improvement in oxidative stress indices (TBARS) implicated the role of angiotensin II signaling through AT1 receptors in the generation of oxidative stress and functional changes in the kidney with hypercholesterolemia.[34]

Cheng et al.[23] studied the interaction of dietary sodium intake and AT1 receptor blockade in spontaneously diabetic rats that develop kidney disease. A high-sodium diet markedly increased 8-iso prostaglandin $F_{2\alpha}$ excretion. Administration of valsartan, an AT1 receptor blocker, to rats given a high-sodium diet improved inflammation, oxidative stress, and albuminuria without affecting BP or endothelial dysfunction. However, when valsartan was given with a low-sodium diet, endothelial dysfunction, albuminuria, oxidative stress, and inflammation were all improved, and there was a reduction in BP. Whereas modulation of dietary sodium did not affect AT1 receptor expression, valsartan administration reduced AT1 receptor expression in cortex and medulla regardless of the dietary sodium intake.[23] This study underscores the important and complex interactions among dietary sodium, angiotensin II effects, AT1 receptor blockade, and oxidative stress.

In a model of diabetic nephropathy, Onozato et al.[35] studied rats after 2 weeks of streptozotocin-induced diabetes mellitus. Rats were randomized to receive no treatment, an angiotensin-converting enzyme (ACE) inhibitor, or an angiotensin AT1 receptor blocker for 2 weeks. At 4 weeks, immunoreactive expression of p47phox (a subunit of NADPH oxidase), endothelial nitric oxide synthase, lipid peroxides, renal hydrogen peroxide production, and nitrotyrosine deposition were increased in the diabetic rats. Treatment with either ACE inhibitor or AT1 receptor blockade prevented all these findings and also prevented the development of significant microalbuminuria. These treatments did not affect the hyperglycemia, BP, or creatinine clearance. These findings indicates a pathogenic role for angiotensin II in the development of oxidative damage in the kidneys during early diabetes mellitus that can be ameliorated by ACE inhibitor therapy or AT1 receptor blockade.[35]

Jones et al.[36] reported that there was a 6.3-fold increase in monocyte chemotactic protein-1 (MCP-1) expression compared with sham-operated rats in a unilateral ureteral obstruction model. MCP-1 expression was markedly reduced by enalapril or losartan treatment. Similarly, Fas expression (an apoptosis effector gene) was increased in the obstructed kidney and was not reduced by enalapril or losartan. The induction of Fas ligand, however, which was upregulated in the obstructed kidney, was attenuated by enalapril or losartan. The expression of apoptotic and chemokine genes that are significantly upregulated in ureteral obstruction can be under the control of redox-sensitive pathways. The attenuation by ACE inhibition and AT1 receptor blockade suggests that angiotensin II plays a central role in the renal damage that occurs with ureteral obstruction.[36]

In a model of cyclosporine nephrotoxicity, Padi et al.[37,38] have shown that oxidative stress indices (TBARS) are reduced by concomitant AT1 receptor blockade with candesartan, and that there was significant protection of renal function and morphology. Cyclosporine and tacrolimus induce a comparable oxidative stress in kidney transplant patients with posttransplant hypertension. Treatment with ramipril normalized BP and reduced the oxidative stress induced by both drugs in these patients.[39]

De Cavanagh et al.[40] have studied the mechanism of reduced aging in rats treated with enalapril or losartan. They found reduced age-related mitochondrial dysfunction and ultrastructural alteration in animals treated with renin–angiotensin system blockers compared with controls, and implicate an

important role for oxidative stress at the mitochondrial levels that is modulated by angiotensin II.

Studies discussed here implicating the role of AT1 receptor-mediated oxidative stress in the kidney are summarized in Table 2.

IN VITRO STUDIES OF THE CELLULAR EFFECTS OF ANGIOTENSIN II

The mechanism of angiotensin II-induced hypertrophy of proximal tubules involves reactive oxygen species and has recently been described by Hannken et al.[41] Activation of membrane-bound NADPH oxidase generates reactive oxygen species that triggers downstream effects, including phosphorylation of p44/42 MAP kinase, stimulation of $p27_{kip1}$ an inhibitor of cyclin-dependent kinase that leads to G1 phase arrest, and proximal tubular hypertrophy. It appears that NOX1 is the isoform of NADPH oxidase that is responsible for angiotensin II-induced oxidative stress.[19]

Lodha et al.[42] have reported that angiotensin II promotes apoptosis of mouse mesangial cells in a dose-dependent manner. This effect of angiotensin II was associated with reactive oxygen species production. AT1 as well as AT2 receptor antagonists attenuated the proapoptotic effect of angiotensin II. Maneuvers that increased HO-1 activity prevented angiotensin II-induced apoptosis. Inhibition of HO-1 enhanced the proapoptotic effect of angiotensin II[42] Haugen et al.[12] have reported that HO-1 activity can be induced by angiotensin II in a cultured renal epithelial cell system. Thus, angiotensin II and oxidative stress play an important role in mesangial cell apoptosis, and the induction of HO-1 appears to protect the cells against these effects.

Renal glomerular epithelial cells (podocytes) play a central role in formation of the glomerular filtration barrier and are intimately involved in the pathogenesis of proteinuric states.[43] Podocyte function is affected by angiotensin II, acting through AT1 receptors.[44] Oxidative stress has been shown to be acutely increased in the puromycin model of nephrotic syndrome,[45,46] and angiotensin II can enhance this effect on podocyte injury. Captopril has been shown to be slightly protective against tissue injury in this model,[47] but the effects of more specific AT1 antagonists have not yet been examined in puromycin nephrotoxicity. Angiotensin II infusion in rats can induce marked renal injury manifested by proteinuria, glomerular phenotypic changes (mesangial expression of alpha-actin and podocyte expression of desmin), and tubulointerstitial injury with the tubular upregulation of the macrophage-adhesive protein, osteopontin, the interstitial accumulation of macrophages and myofibroblasts, and the deposition of collagen types III and IV.[48] Losartan, but not ramipril, completely blocked the angiotensin II-mediated hypertension, proteinuria, and renal injury.[48] The role of oxidative stress in this model of angiotensin II-mediated renal injury has not yet been examined.

Keidar et al.[49] studied mouse peritoneal macrophages from apolipoprotein-E-deficient mice that demonstrate an age-dependent increase in cellular lipid–peroxide content and AT1 receptor mRNA expression. Vitamin E supplementation significantly decreased the cellular lipid–peroxides and macrophage AT1 receptor mRNA expression. Buthionine–sulfoximine, a glutathione synthesis inhibitor, increased cellular lipid–peroxide content and AT1 receptor mRNA expression, whereas L-2-oxothiazolidine-4-carboxylic acid, which contributes to glutathione synthesis, reduced macrophage cellular lipid–peroxide content and AT1 receptor mRNA expression. Incubation of macrophages with oxidized low-density lipoproteins led to a significant increase in macrophage AT1 receptor mRNA and protein expression compared with control cells. These findings implicate a role for oxidative stress in the induction of AT1 receptor expression in macrophages. This phenomenon can stimulate the interaction of angiotensin II with macrophages and hence accelerate macrophage foam cell formation and early atherogenesis. In a human monocytic leukemia cell line, Yanagitani et al.[50] have demonstrated reduced peroxide production with AT1 receptor blockers in a dose-dependent manner, which further supports the role of angiotensin II signaling by AT1 receptors in stimulating oxidative stress.

Angiotensin II induces oxidative stress in endothelial cells. Stimulation of pathways that mitigate oxidative stress can reduce angiotensin II-induced, oxidative stress-mediated DNA damage and apoptosis. Mazza et al.[51] have shown that overexpression of HO-1 reduces angiotensin II-induced DNA damage and mitigates oxidative stress. Furthermore, endothelial activation by angiotensin II can be critically dependent on free radical signaling. Angiotensin II induces intracellular oxidative stress in endothelial cells, which stimulates I-kappaB degradation and NF-kappaB

Table 2. Effects of Angiotensin II in the Kidney Mediated by Oxidative Stress

Reference	Model	Response
Angiotensin II Animal Infusion Models		
Haugen et al.[12]	Angiotensin II infusion	Increase in oxidative stress in the kidney, injury predominantly in the proximal tubule
Kawada et al.[13]	Graded doses of angiotensin II, time course experiment	Early increase in postglomerular resistance that translates into renal vascular constriction and development of hypertension
Nishiyama et al.[14]	Angiotensin II infusion	Quenching of nitric oxide and occurrence of hypertension
Nagase et al.[15]	Angiotensin II infusion	Upregulation of aortic oxidized low-density lipoprotein receptor (LOX-1) blocked by tempol administration
Delbosc et al.[16]	Rat myocardial infarction, congestive heart failure model associated with generation of reactive oxygen species	Hemodynamic function, oxidative stress and left ventricular mass were improved with AT1 receptor blockade
Diep et al.[17]	Angiotensin II infusion	NADPH oxidase activity and vascular wall inflammation were modulated by a peroxisome proliferator-activated receptor-alpha activator
Virdis et al.[18]	Angiotensin II and aldosterone infusion	Aortic NADPH oxidase activity and plasma TBARS activity normalized by spironolactone
Chabrashvili et al.[19]	Angiotensin II infusion	Angiotensin II increased 8-iso prostaglandin $F_{\alpha 2}$; and MDA excretion by 41%; prevented by AT1 receptor blockade, and increased by AT2 receptor blockade
Nonangiotensin II Effects in Transgenic Animal Models		
Mervaala et al.[20]	Double transgenic rats for human renin and human angiotensinogen	Quenching of nitric oxide, generation of oxidative stress and endothelial dysfunction
Wang et al.[21]	gp91$_{phox}$ Knockout mice + angiotensin II infusion	Generation of oxidative stress in areas limited to NADPH oxidase expression
Role of Dietary Salt Intake		
Rugale et al.[22]	Angiotensin II infusion + variation in dietary sodium intake	Sodium restriction mitigates albuminuria, oxidative stress, and occurrence of left ventricular hypertrophy
Cheng et al.[23]	Spontaneous diabetic rat model	Increased dietary sodium intake dramatically increased 8-iso prostaglandin $F_{2\alpha}$ production
Renal Disease Models Associated With Increased Angiotensin II Production		
Lerman et al.[24]	Unilateral renal artery stenosis	Early and persistent increase in lipid peroxidation markers despite quick return of renin to baseline implicating the role of oxidative stress in sustaining hypertension in renovascular hypertension
Welch et al.[25]	Spontaneous hypertensive rat	Angiotensin II-mediated oxidative stress causes reduced efficiency of renal oxygen utilization and impaired nitric oxide signalling in the juxtaglomerular apparatus
Welch et al.[26]	Post-clip one-clip, two-kidney rat	Angiotensin II-mediated oxidative stress causes reduced efficiency of renal oxygen utilization and impaired nitric oxide signalling in the juxtaglomerular apparatus
Welch et al.[27]	Spontaneous hypertensive rat	Exaggerated tubuloglomerular feedback in SHR
Rodriguez-Iturbe et al.[28-31]	Hypertensive rat models	Immune cell infiltration play an important role in hypertensive models, blood pressure normalized and tissue damage minimized by immunosuppression therapy
Bapat et al.[32]	Chronic nitric oxide synthase inhibition	Oxidative stress mediated by angiotensin II and injury predominantly in tubules, not blood vessel
Kitamoto et al.[33]	Chronic nitric oxide synthase inhibition	AT1 receptor blockade reduced aortic superoxide anion production and oxidative stress parameters
Chade et al.[34]	Hypercholestrolemic pigs	Renal hemodynamic and tubular dysfunction mediated by angiotensin II induced oxidative stress
Onozato et al.[35]	Streptozotocin induced diabetes mellitus	Diabetes is associated with an early AT1 receptor-mediated increase in oxidative stress independent of blood pressure, hyperglycemia, or GFR
Jones et al.[36]	Ureteral obstruction	Inflammation and apoptosis in obstructive uropathy is mediated by AT1 receptors likely by redox-sensitive genes
Padi et al.[37,38]	Cyclosporine nephrotoxicity	AT1 receptor-mediated oxidative stress is increased with cyclosporine nephrotoxicity
Calo et al.[39]	Cyclosporine and tacrolimus nephrotoxicity	Ramipril reduced hypertension and reduced oxidative stress indices
de Cavahagh et al.[40]	Aging in rats	Enalapril and losartan reduced mitochondrial oxidative stress associated with aging

Abbreviations: NADPH, nicotinamde adenine dinucleotide phosphate; TBARS, thiobarbituric acid reactive substances; MDA, malonyl-dialdehyde; GFR, glomerular filtration rate.

activation. Pueyo et al.[52] studied the endothelial expression of vascular cell adhesion molecule-1 (VCAM-1) by angiotensin II. Angiotensin II stimulated mRNA and protein expression of VCAM-1 in these cells through the AT1 receptor, and this effect was blocked by pyrrolidinedithiocarbamate, an antioxidant molecule. Angiotensin II activated the redox-sensitive transcription factor NF-kappaB. Inhibitors of reactive oxygen species generated in the mitochondria reduced angiotensin II-induced I-kappaB degradation. A glutathione peroxidase mimic inhibited the effect of angiotensin II, and an inhibitor of catalase enhanced it, suggesting a role for H_2O_2 in I-kappaB degradation. This activation enhances the expression of VCAM-1 causing endothelial activation and could be involved in the early stages of atherosclerosis.[52]

Angiotensin II causes proliferation of vascular smooth muscle cells and enhanced DNA synthesis. Mueller et al.[53] have identified a novel redox-sensitive gene, the dominant-negative helix–loop–helix protein Id3, which is upregulated within 30 minutes by xanthine oxidase and angiotensin II, revealing a novel redox-sensitive pathway involved in growth of these cells. Overexpression of sense Id3 downregulated protein expression of $p21_{WAF1/Cip1}$, $p27_{Kip1}$, and p53, whereas overexpression of antisense Id3 abrogated the effect of angiotensin II on the expression of $p21_{WAF1/Cip1}$, $p27_{Kip1}$, and p53. Hyperphosphorylation of the retinoblastoma protein was seen with angiotensin II and overexpression of sense Id3 and was mitigated by overexpression of antisense Id3. Thus, angiotensin II induces proliferation of vascular smooth muscle cells by production of superoxide, which enhances the expression of Id3. Id3 governs the downstream mitogenic processing through suppression of $p21_{WAF1/Cip1}$, $p27_{Kip1}$ and p53. Schieffer et al.[54] have demonstrated in rat aortic smooth muscle cells that stimulation of the JAK/STAT cascade by angiotensin II requires superoxide anions generated by the NAD(P)H oxidase system and is involved in angiotensin II-induced IL-6 synthesis. Thus, the post-AT1 receptor signaling and transcription events that transduce the effects of angiotensin II on oxidative stress and the inflammatory state are beginning to be unraveled.

HUMAN STUDIES ON ANGIOTENSIN II AND OXIDATIVE STRESS INDUCTION

Higashi et al.[55] studied patients with renovascular hypertension who have an activated renin–an-giotensin system. Endothelial function was evaluated by the response of forearm blood flow to acetylcholine, an endothelium-dependent vasodilator, and isosorbide dinitrate, an endothelium-independent vasodilator, before and after renal artery angioplasty in 15 subjects with renovascular hypertension and 15 control subjects without hypertension who were matched for age and sex. The forearm blood flow in response to acetylcholine was less in subjects with renovascular hypertension than in control subjects, although the forearm blood flow in response to isosorbide dinitrate was similar in the two groups. After renal angioplasty, the forearm blood flow in response to acetylcholine was increased by 53%. Angioplasty decreased BP (systolic and diastolic), forearm vascular resistance, and urinary excretion of 8-hydroxy-2′-deoxyguanosine and serum malondialdehyde-modified low-density lipoprotein (LDL), indices of oxidative stress. Furthermore, the increase in the maximal forearm blood flow in response to acetylcholine correlated significantly with the decrease in urinary excretion of 8-hydroxy-2′-deoxyguanosine and serum malondialdehyde-modified LDL. Coinfusion of ascorbic acid (vitamin C), an antioxidant, augmented the response of forearm blood flow to acetylcholine before angioplasty but not after angioplasty. Taken together, these findings suggest that excessive oxidative stress is involved, at least in part, in impaired endothelium-dependent vasodilatation in patients with renovascular hypertension, perhaps as a result of systemic effects of angiotensin II, and that these effects are resolved by successful angioplasty.[55]

Further support for the role of angiotensin II in causing lipid peroxidation comes from studies of Minuz et al.[56] who measured the urinary excretion of 8-iso-prostaglandin $F_{2\alpha}$ in 25 patients with renovascular disease, 25 patients with essential hypertension, and 25 healthy subjects. Urinary 8-iso prostaglandin $F_{2\alpha}$ was significantly higher in patients with renovascular disease than in patients with essential hypertension or in healthy subjects, and correlated with renal vein renin and angiotensin II ratios. Patients were also studied 6 months after technically successful angioplasty of their renal artery stenosis. A reduction in 8-iso prostaglandin $F_{2\alpha}$ excretion after angioplasty was observed in those with renovascular disease who had high baseline levels of lipid peroxidation. These results show that enhanced lipid peroxidation in hypertensive patients with renovascular disease is

related to activation of the renin–angiotensin system and is presumably mediated by the systemic effects of angiotensin II and enhanced oxidative stress.

Hypercholesterolemia causes upregulation of AT1 receptors, which could be a key event in the development of endothelial dysfunction.[8] Wassmann et al.[57] studied the effect of a 6-week treatment with candesartan on endothelial function and serum inflammation markers and compared its effect with placebo or felodipine in 47 hypercholesterolemic patients. Forearm blood flow by venous occlusion plethysmography during reactive hyperemia was significantly improved by candesartan, whereas felodipine and placebo exerted no effect independent of BP or serum cholesterol. Serum concentrations of the oxidative marker 8-iso PGF$_{2\alpha}$ and inflammatory markers MCP-1 and soluble intercellular adhesion molecule-1 were significantly reduced by candesartan treatment but not by placebo or felodipine. These data suggest that AT1 receptor antagonism of the effects of angiotensin II improves endothelial function during hypercholesterolemia, and that this effect is associated with reduced oxidative stress and reduced endothelial activation.

Annuk et al.[58] studied the relationship between oxidative stress and endothelium-dependent vasodilation in 37 patients with chronic kidney disease using plethysmographic evaluation of endothelial function. Impaired endothelium vasodilation function and oxidative stress were related in these patients, but the role of angiotensin II has not yet been evaluated.

Touyz et al.[59] have demonstrated that the enhanced oxidative stress and growth-promoting actions of angiotensin II are associated with increased activation of phospholipase D (PLD)-dependent pathways. They studied the relationship between responsiveness to angiotensin II, oxidative stress, and growth responses in vascular smooth muscle cells from untreated essential hypertensive patients and normotensive control subjects. Angiotensin II increased H$_2$O$_2$ generation significantly more in hypertensives compared with normotensives. Angiotensin II increased PLD activity and DNA and protein synthesis in cells from hypertensive more than normotensive subjects. PLD inhibition attenuated angiotensin II-induced reactive oxygen species generation, with greater effects in the hypertensive group than the normotensive group. These processes could contribute to

vascular remodeling in hypertension. Lowering of BP in patients with essential hypertension also improves the oxidation of LDL cholesterol.[60] Concomitant therapy with an AT1 receptor blocker (valsartan) and a statin (fluvastatin) therapy in seven patients with hypercholesterolemia and hypertension without kidney disease reduced the propensity for in vitro low-density lipoprotein oxidation by 17% and reduced oxidative stress indices (TBARS) by 21%.[61]

The underlying mechanism of the proinflammatory effects of angiotensin II could be more complex than signaling by the AT1 receptor and increased NADPH oxidase activity. It has recently been recognized that there could be angiotensin II-independent effects of AT1 receptor antagonists, especially on thromboxane receptor binding.[62-65] Recently, Kramer et al.[66] described a metabolite of losartan (EXP3179) that shows molecular homology to indomethacin. Serum-levels of EXP3179 were measured in patients receiving a single oral dose of 100 mg losartan. The peak level of EXP3179 was 10^7 M between 3 to 4 hours and was associated with a significant reduction in platelet aggregation in vivo. When human endothelial cells were exposed to angiotensin II or lipopolysaccharides (LPS) in the presence of 10^7 M EXP3179, LPS- and angiotensin II-induced COX-2 transcription was abolished. Moreover, EXP3179 significantly reduced angiotensin II- and LPS-induced formation of prostaglandin F$_{2x}$. Thus, the antiinflammatory properties of losartan can also be mediated by the inhibitor effect of EXP3179 on COX-2 mRNA transcription and subsequent COX-dependent thromboxane A$_2$ and prostaglandin F$_2$ generation. Whether this effect is mediated by the inhibitory effects of losartan and/or its metabolites on the thromboxane receptors[65,67,68] or on COX-2 transcription has not been defined. The AT1 receptor antagonist-specific metabolite of losartan (EXP3174) has very little effect on thromboxane receptor binding,[67] so that the inhibitory effect of losartan on thromboxane receptor binding must be mediated by another metabolite. Irbesartan has also been described as having inhibitory effects on the thromboxane receptor, but the effects of other AT1 receptor blockers on these receptors or COX-2 transcription are not as prominent or have not yet been defined.[63,68-70]

Agarwal[5] examined the influence of added AT1 receptor blockade with losartan (50 mg/day) on oxidative stress and the proinflammatory state of

the kidney in patients with chronic kidney disease who had been chronically treated with maximal doses of an ACE inhibitor (40 mg lisinopril per day) as well as other antihypertensive agents. Oxidative stress to proteins was measured by an high-pressure liquid chromatography assay for carbonyl concentration. Urinary inflammation was measured by MCP-1 excretion rate. There was no change in proteinuria or 24-hour ambulatory BP when losartan therapy was added. Before losartan therapy, urinary protein and albumin oxidation were 99% and 71% higher, respectively, compared with plasma proteins and albumin, demonstrating that these proteins were exposed to an oxidative environment in the urinary space. There was a highly significant 35% reduction in urinary oxidized albumin when losartan therapy was added to the ACE inhibitor/antihypertensive treatment. Thus, in proteinuric patients, urinary albumin can serve as a target for and index of oxidative injury as it passes from the glomerulus to the urine. Urinary and plasma MDA were elevated compared with age-matched control subjects. A good correlation was seen between the change in urinary oxidized albumin and MCP-1 levels (r = 0.61, P = 0.012). These data demonstrate that oxidative damage to urinary protein can be reduced with additional AT1 receptor blockade, independently of reductions in proteinuria or BP. Urinary measurements of markers of oxidative damage to proteins could be a more sensitive index of renal oxidative stress than plasma measurements in patients with chronic kidney disease. The significant association of the change in urinary MCP-1 with a reduction in oxidative stress is consistent with a role of the redox state in the kidney as a mediator of fibrosis and progressive tissue damage in chronic kidney disease.[5]

SUMMARY

Angiotensin II causes oxidative stress in the kidney through the AT1 receptor in many cell types and mediates endothelial dysfunction, renal hemodynamic changes, and generation of hypertension, inflammation, apoptosis, and progressive renal damage. Direct inhibitory effects of AT1 antagonists and/or their metabolites on thromboxane receptors and COX-2 activity could also play a role in the anti-inflammatory and antiaggretory effects of AT1 receptor blockers. A high-sodium diet can further compound the damage associated with angiotensin II by generating further oxidative stress. Generalized endothelial dysfunction can be observed when the production of angiotensin II is localized to the kidneys, like in renovascular hypertension. Although ACE inhibitors and AT1 receptor blockers are attractive drugs for mitigation of angiotensin II-mediated oxidative stress, direct antagonism of oxidative stress with new dietary or pharmaceutical agents appears to be an attractive opportunity for the treatment of patients with hypertension, atherosclerosis, and chronic kidney disease.

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