Oxidants in Chronic Kidney Disease

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Chronic kidney disease is a worldwide public health problem that affects approximately 10% of the US adult population and is associated with a high prevalence of cardiovascular disease and high economic cost. Chronic renal insufficiency, once established, tends to progress to end-stage kidney disease, suggesting some common mechanisms for ultimately causing scarring and further nephron loss. This review defines the term *reactive oxygen metabolites* (ROM), or oxidants, and presents the available experimental evidence in support of the role of oxidants in diabetic and nondiabetic glomerular disease and their role in tubulointerstitial damage that accompanies progression. It concludes by reviewing the limited human data that provide some proof of concept that the observations in experimental models may be relevant to human disease.

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Chronic kidney disease (CKD) is a worldwide public health problem that affects approximately 10% of the US adult population (1) and is associated with a high prevalence of cardiovascular disease (2) and high economic cost (3,4). Chronic renal insufficiency, once established, tends to progress to end-stage kidney disease, even when the primary disease process has been treated or seems to be inactive. This suggests that alterations and adaptations in nephrons that remain after the initial insult ultimately cause scarring and further nephron loss, which result in an end-stage kidney.

The notion that oxidants may be important in inflammation and tissue injury first was suggested by Joe McCord, a graduate student with Dr. Irwin Fridovich at Duke University, who in 1969 described the enzyme superoxide dismutase. A small pharmaceutical company in California, Diagnostic Data, previously had isolated the protein as a naturally occurring anti-inflammatory protein but had no knowledge of its enzymatic activity. They labeled this protein orgotein and quickly recognized its identity with superoxide dismutase on the basis of the physical and spectral characteristics. A key piece of information that neutrophils produce superoxide anion became available (5). As McCord stated, "For me, it suddenly became clear." He reasoned that, because phagocytosing neutrophils, the effector cells of the acute inflammatory response, release large amounts of superoxide extracellularly and superoxide dismutase, an enzyme that scavenges superoxide, possesses anti-inflammatory activity, superoxide anion and other oxygen metabolites could be important mediators of the inflammatory process (6,7). In this review, we define the term *reactive oxygen metabolites* (ROM), or oxidants, and present the available experimental evidence in support of the role of oxidants in diabetic and nondiabetic glomerular disease and their role in tubulointerstitial damage that accompanies progression. We conclude by reviewing the limited human data that provide some proof of concept that observations in experimental models may be relevant to human disease.

ROM

Partial reduction of oxygen can and does occur in biologic systems, which leads to the generation of partially reduced and potentially toxic reactive oxygen intermediates (8,9). Sequential reduction of oxygen along the univalent pathway leads to the generation of superoxide anion, H_2O_2, hydroxyl radical, and water (8,9).

$$\text{Oxygen} \rightarrow \underset{\text{(free radical)}}{\text{superoxide}} \rightarrow \text{hydrogen peroxide} \rightarrow \underset{\text{(free radical)}}{\text{hydroxyl radical}} \rightarrow \text{water}$$

The metabolism of H_2O_2 by neutrophil-derived myeloperoxidase (MPO; the enzyme that is responsible for the green color of pus) generates highly reactive toxic products, including hypochlorous acid (HOCl, the active ingredient in Clorox bleach).

$$H_2O_2 + \text{CT MPO HOCl} + H_2O \longrightarrow$$

At least one functional role for HOCl, that of antimicrobial activity, has been well established. The various products that result from the MPO-H_2O_2-chloride system, including tyrosyl radical adduction products, as well as their potential role in kidney disease, are detailed in a review by Malle *et al.* (10).

Superoxide and H_2O_2 seem to be the primary species generated; they may play a role in the generation of additional and more reactive oxidants, including the highly reactive hydroxyl radical (or a related highly oxidizing species), in which metals such as iron act as a catalyst in a reaction that commonly is

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referred to as the metal-catalyzed Haber-Weiss reaction (11).

$$Fe^{3+} + O_2^{\cdot} \longrightarrow Fe^{2+} + O_2$$

$$Fe^{2+} + H_2O_2 \longrightarrow Fe^{3+} + OH^- + OH^{\cdot}$$

$$O_2 + H_2O_2 \qquad O_2 + OH^- + OH^{\cdot}$$

Iron, a transitional metal, can serve as a carrier for oxygen and electrons and as a catalyst for oxygenation, hydroxylation, and other critical metabolic processes, in part because of its ability to cycle reversibly and readily between the ferrous and ferric oxidation states. The ease with which iron is oxidized and reduced reversibly is essential for its metabolic functions. However, this property makes iron potentially hazardous by enabling it to participate in the generation of powerful oxidant species such as hydroxyl radical and/or reactive iron–oxygen complexes such as ferryl or perferryl ion (11).

Iron also has a major role in the initiation and propagation of lipid peroxidation, by either catalyzing the conversion of primary oxygen radicals to hydroxyl radicals or forming a perferryl ion. In addition, iron can catalyze directly lipid peroxidation, the oxidative reaction of polyunsaturated lipids, by removing hydrogen atoms from polyunsaturated fatty acids in the lipid bilayers of organelle membranes (11).

Because iron can participate in the formation of ROM, organisms take great care in the handling of iron, using transport proteins such as transferrin and storage proteins such as ferritin and minimizing the size of the intracellular iron pool. The availability of iron to stimulate hydroxyl generation *in vivo* is very limited under normal conditions; this iron sequestration may be regarded as a contribution to antioxidant defenses. Although there has been much debate about the availability of catalytic metal ions *in vivo*, it now is well established that oxidant stress itself can provide catalytic iron (11). These oxygen metabolites, including the free-radical species superoxide and hydroxyl radical, and other metabolites, such as H_2O_2 and hypohalous acids, often are referred to collectively as ROM, or reactive oxygen species (ROS), or simply as oxidants.

Experimental Studies

Role of Oxidants in Leukocyte-Dependent Glomerulonephritis

The two models of proliferative glomerulonephritis that have been well studied are the anti–glomerular basement membrane (anti-GBM) antibody model and anti–Thy 1.1. The anti-GBM antibody is a well-characterized model of complement- and neutrophil-dependent glomerular injury, and anti–Thy 1.1 is a well-characterized model of mesangioproliferative glomerulonephritis, which is induced by an anti–mesangial cell antibody. In this section, we review the evidence for enhanced generation of oxidants, the ability of oxidants to cause proteinuria, and studies with scavengers of ROM (Table 1).

Enhanced generation of oxidants has been demonstrated in anti–Thy 1.1 and anti-GBM–induced glomerulonephritis with cytochemical techniques (12) and in isolated glomeruli (13) or macrophages (13,14). Several immune reactants, such as serum-treated zymosan (a C_3b receptor stimulus), heat-aggregated IgG (an Fc receptor stimulus), immune complexes, complement components, and antinuclear antibody (15) all have been shown to trigger the oxidative burst. This suggests that oxidants may

Table 1. Evidence for the role of oxidants in leukocyte-dependent GN[a]

Leukocytes as a source of oxidants for glomerular injury
- a wide variety of soluble and particulate stimuli, including immune complexes, complement components (26), and ANCA (15)
- anti-GBM enhances generation of oxidants by neutrophils *in vitro*
- cytochemical detection of the presence of superoxide- and H_2O_2-generating leukocytes in anti–Thy 1.1 and anti-GBM–induced GN (12)
- enhanced superoxide and hydroxyl radical are generated by macrophages that are isolated from glomeruli of rabbits with anti-GBM antibody disease (14)
- enhanced superoxide generation by macrophages that are isolated from nephritic glomeruli (anti-thymocyte serum) (125)

Effects of oxidants that are relevant to occurrence of proteinuria in glomerular injury
- oxidants participate in GBM degradation (16)
- infusion of phorbol myristate acetate, an activator of neutrophils, results in proteinuria and a fall in GFR. These effects are prevented by a catalase.
- infusion of myeloperoxidase-H_2O_2 induces proteinuria (20)

Evidence for the role of oxidants in animal models
- catalase markedly reduces proteinuria, whereas superoxide dismutase has no protective effect in anti-GBM antibody disease (23)
- a hydroxyl radical scavenger and an iron chelator significantly attenuate anti-GBM antibody-induced proteinuria in anti-GBM antibody disease (24)
- α-lipolic acid is protective in an anti–Thy 1.1 model (25)

[a]GBM, glomerular basement membrane; GN, glomerulonephritis.

be important in exudative and proliferative glomerulonephritis.

Leukocytes can cause proteinuria (a hallmark of glomerular diseases) by damaging the GBM. The degradation of the GBM by stimulated neutrophils is caused by the activation of a latent metalloenzyme (most likely gelatinase) by HOCl or a similar oxidant that is generated by the MPO-H_2O_2-halide system (16). In addition to this *in vitro* observation, infusion of phorbol myristate acetate (a potent activator of leukocytes) or of cobra venom factor in the renal artery caused significant proteinuria that was prevented by catalase (which destroys H_2O_2) and neutrophil depletion (17–19). Infusion of MPO followed by H_2O_2 results in significant proteinuria (20) and, 4 to 10 d later, development of a marked proliferative glomerular lesion (21). In addition to causing proteinuria, it has been shown that an oxidant-generating system induces a reduction in the glomerular and mesangial cell planar surface and an increase in myosin light-chain phosphorylation, a biochemical marker of contraction (22), which, by decreasing the surface area of mesangial cells, could result in a decrease in GFR.

In an anti-GBM antibody model, treatment with catalase markedly reduced proteinuria (23), and a hydroxyl radical scavenger or iron chelator significantly attenuated proteinuria (24). In an anti–Thy 1.1 model, treatment with antioxidant α-lipolic acid resulted in reduced generation of oxidants, reduced phosphorylated extracellular signal–regulated kinase (ERK), significant improvement in glomerular injury as measured histologically, and reduced expression of TGF-β1 (25).

Animal Model of Minimal-Change Disease

The ability of glomerular cells to generate oxidants suggests that they may be important mediators of glomerular injury in glomerular diseases that lack infiltrating leukocytes. An animal model of minimal-change disease is induced by a single intravenous injection of puromycin aminonucleoside (PAN). In this section, in addition to previous evidence (26), we will provide additional support for the role of oxidants in this model. PAN enhances the generation of superoxide anion, H_2O_2, and hydroxyl radical (27,28). Several studies have shown that scavengers of ROM and iron chelators reduce proteinuria in this model (29–32). Bleomycin-detectable iron (iron that is capable of catalyzing free-radical reactions) was increased markedly in glomeruli from nephrotic rats, and an iron chelator prevented an increase in catalytic iron in glomeruli and provided complete protection against proteinuria, suggesting an important pathogenic role for glomerular catalytic iron in this model (33). Baliga *et al.* (34) recently demonstrated that cytochrome P450—more specific, cytochrome P450 2B1, an isozyme that is present in the glomerulus—is a source of catalytic iron that participates in glomerular injury in this model (35,36) (Table 2).

Several other lines of evidence support a role for ROM in this model. Glutathione peroxidase is a selenoenzyme that catalyzes the reduction of H_2O_2 to water. Feeding rats a selenium-deficient diet results in marked diminution of glutathione peroxidase and is accompanied by a marked increase in urinary protein after PAN injection, suggesting an important role of glutathione peroxidase in this model of glomerular disease (37). Similarly, inhibition of superoxide dismutase augments PAN-induced proteinuria (38). These studies not only demonstrate the importance of endogenous antioxidant defenses but also provide additional support for the role of ROM in these models of glomerular injury.

Animal Model of Membranous Nephropathy

Passive Heymann nephritis, induced by a single intravenous injection of anti-Fx1A, is a complement-dependent model of glomerular disease that resembles membranous nephropathy in humans. Although leukocytes have not been considered to be important pathogenetically in animal models of membranous nephropathy, there is evidence for the potential participation of an MPO-H_2O_2-chloride system in membranous nephropathy (10,39). Thus it appears that leukocytes or resident

Table 2. Evidence for the role of oxidants in leukocyte-independent GN[a]

In a PAN model of minimal-change disease
- cultured glomerular epithelial cells exhibit an enhanced generation of H_2O_2 (28)
- administration of scavengers of oxidants and antioxidants results in reduction in proteinuria (29–32)
- glomerular catalytic iron increases (33), and cytochrome P450 is an important source of the catalytic iron (34–36)
- feeding a selenium-deficient diet results in a marked diminution of glutathione peroxidase accompanied by an increase in proteinuria (37)
- inhibition of superoxide dismutase by diethyldithiocarbamate results in increase in PAN-induced proteinuria (38)
- induction of antioxidant enzymes by ischemia-reperfusion injury protects against H_2O_2-induced proteinuria (126)
- induction of antioxidant enzymes by glucocorticoids protects against PAN-induced proteinuria (127)

Evidence for the role of oxidants in passive Heymann nephritis
- there is an increased generation of H_2O_2 in passive Heymann nephritis (40)
- in a passive Heymann nephritis model of membranous nephropathy, hydroxyl radical scavengers and an iron chelator and probucol significantly reduce proteinuria (41,46)
- feeding an iron-deficient diet results in a reduction in proteinuria
- feeding a selenium-deficient diet results in marked diminution of glutathione peroxidase in anti-Fx1A–induced proteinuria (44)

[a]PAN, puromycin aminonucleoside.

glomerular cells serve as sources for oxidants in this model. In an *in vivo* study using cytochemical techniques, it has been shown that there is increased generation of H_2O_2 in passive Heymann nephritis (40) (Table 2).

The administration of scavengers of hydroxyl radical or iron chelator markedly reduces proteinuria, suggesting the role of hydroxyl radical in passive Heymann nephritis (41) and in the cationized γ-globulin–induced immune complex glomerulonephritis, another model of membranous nephropathy (42). Baliga *et al.* (43) showed that feeding an iron-deficient diet provides protection in this model and that rats that are on a selenium-deficient diet have decreased glutathione peroxidase activity and a worsening of proteinuria (44). In addition, probucol, an inhibitor of lipid peroxidation, reduces proteinuria (45).

Oxidant Mechanisms in Diabetes

A large body of evidence indicates that diabetes is a state of increased oxidative stress, and it has been suggested that oxidants are the causative link for the major pathways that have been implicated in vascular complications of diabetes (46,47). The evidence for the role of oxidants in diabetic nephropathy includes the following: High glucose increases production of oxidants in glomerular cells, oxidants have direct biologic effects that are relevant to diabetic nephropathy, and antioxidants reduce the high glucose–induced biologic effects (Table 3).

In this section, we emphasize some of the recent developments, including the pathways that are responsible for enhanced generation of oxidants, the relation between angiotensin II (AngII) and oxidants, and some of the newer mechanisms for diabetic nephropathy in which oxidants play an important role. In *in vitro* studies, it has been shown that high glucose (10 to 30 mM) results in increased generation of oxidants by mesangial cells (48–50). In *in vivo* studies, glomeruli that were isolated from diabetic rats had increased production of superoxide and H_2O_2 (51,52). In addition to the direct effect of high glucose, advanced glycation end products (AGE) bind to receptors for AGE and initiate oxidant production (46,53). Indeed, AGE have been shown to increase intracellular generation of ROM in mesangial cells (54).

There are potentially several pathways for enhanced generation of oxidants. Phagocyte-like NAD(P)H oxidase is a major source of oxidants in many nonphagocytic cells, including renal cells such as tubular epithelial cells and glomerular mesangial cells. These NAD(P)H oxidases are isoforms of the neutrophil oxidase, in which the catalytic subunits, termed Nox proteins,

Table 3. Oxidants in diabetic nephropathy[a]

In vitro studies
- high glucose results in
  - ROS generated by mesangial cells (48,50,62)
  - activation of NAD(P)H oxidase (56,58,95)
  - lipid peroxidation in isolated glomeruli, which is prevented by hydroxyl radical scavengers
- antioxidants prevent glucose-induced
  - activation of PKC and NK-κB (62)
  - upregulation of TGF-β1 (63,68)
  - upregulation of fibronectin (63)
  - upregulation of endothelin-1 (52)
- oxidants cause apoptosis of podocytes (74) and mesangial cells (75)

In vivo studies
- glomeruli isolated from diabetic rats have increased production of oxidants including superoxide and H_2O_2 (51,52,54)
- antioxidants prevent functional and morphologic changes of diabetes (54,64,65,67–71)
- *in vivo* inhibition of Nox4 by antisense reduced whole-kidney and glomerular hypertrophy accompanied by reduced expression of fibronectin protein (95)
- selenium-deficient diet causes an increase in albuminuria, glomerular sclerosis in diabetic rats, and an increase in TGF-β1 (72)

Human studies
- increase in the 8-oxodG content, a marker of oxidative stress, and urine in patients with type 1 (insulin-dependent) and type 2 (non–insulin-dependent) diabetes (92–94)
- high levels of 8-iso PGF2 (128), which is a widely recognized marker of oxidative stress
- patients with diabetic complications had higher levels of 8-oxodG content than those without complications (94)
- progression of diabetic nephropathy in patients with a higher excretion of 8-oxodG (96)
- 8-OHdG was significantly higher in patients with albuminuria (97)
- high urinary catalytic iron in patients with diabetic nephropathy
- diabetic nodular lesions in humans stain positive for malondialdehyde (102)

[a]8-iso PGF2, F2 isoprostane 8-iso prostaglandin F2; 8-oxodG, 8-Oxo-7,8-dihydro-2′-deoxyguanosine; PKC, protein kinase C; ROS, reactive oxygen species.

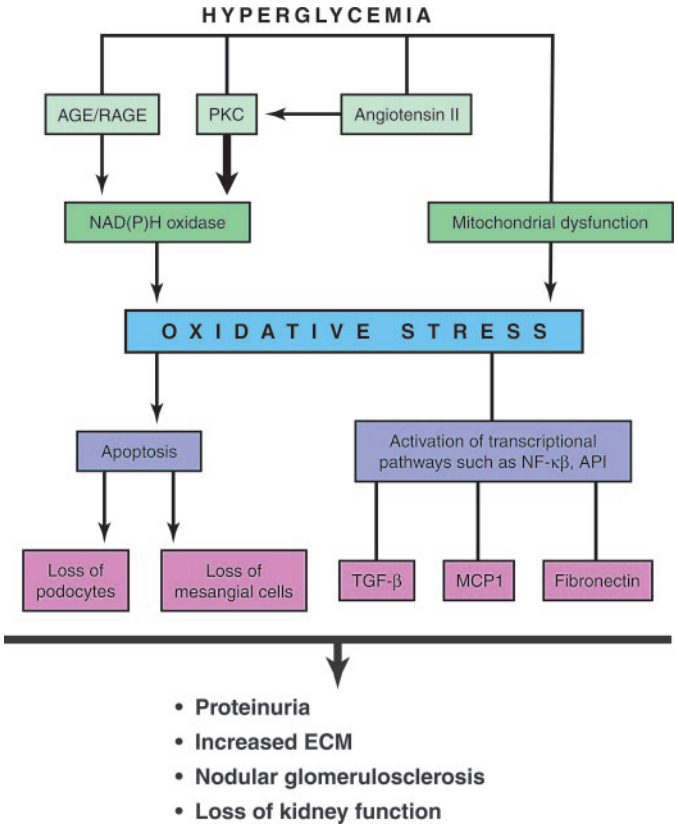

Figure 1. Relation between oxidant stress and various pathways that have been implicated in diabetic nephropathy. Illustration by Josh Gramling—Gramling MedicalIllustration.

correspond to homologues of gp91^{phox} (or Nox2), the catalytic moiety found in phagocytes. Recent studies suggest the important role of NAD(P)H oxidase (55) and protein kinase C (PKC) (49,50) in enhanced oxidant production in diabetes. Nox4, which seems to share the same overall structure with gp91^{phox}/Nox2, is abundant in the vascular system, kidney cortex, and mesangial cells. Gorin *et al.* (56) showed that Nox4 protein expression is increased in the diabetic kidney cortex and that the administration of antisense inhibited NAD(P)H-dependent oxidant generation in the renal cortex, glomeruli, and cultured mesangial cells. Mitochondrial metabolism also has been suggested as an important source for the generation of oxidants in response to high glucose (48), similar to that proposed in the vascular bed (46,47).

There is evidence that the renoprotective effects of Ang II type 1 receptor blockers (ARB) and angiotensin-converting enzyme inhibitors (ACEI), independent of lowering BP, may be related to the effects on oxidant stress (57). It has been shown that incubation of mesangial cells in high glucose results in an increase in Ang I and Ang II levels and an increase in superoxide, which is mediated through the NAD(P)H oxidase system (58). Izuhara *et al.* (59) showed that ARB but not calcium channel blockers inhibited hydroxyl radical–mediated o-tyrosine formation and transition metal–catalyzed oxidation.

Oxidants can activate in mesangial cells most of the known pathways that have been implicated in diabetes (49), including PKC (49), mitogen-activated protein kinases (MAPK), TGF-β1 (60), and fibronectin (60,61). Structurally different antioxidants suppress high glucose–induced PKC activation in rat mesangial cells (62), proximal tubular cells (63), and glomeruli of streptozotocin-induced diabetic rats (64,65). Antioxidants also prevent upregulation of TGF-β1 (62,66) and fibronectin (62) and activation of transcription factors NF-κB and AP-1 in mesangial cells (61). Ha *et al.* (61) showed that high glucose rapidly activates NF-κB in mesangial cells through PKC and oxidants, resulting in upregulation of monocyte chemoattractant protein-1 (MCP-1) mRNA and protein expression. The higher endothelin (ET-1) in glomeruli that are isolated from diabetic rats is attenuated markedly by ROM scavengers as well as an iron chelator (51). Gorin *et al.* (56) showed that *in vivo* inhibition of Nox4 by antisense reduced whole-kidney and glomerular hypertrophy, accompanied by reduced expression of fibronectin protein and Akt/protein kinase B and ERK1/2, two protein kinases that are critical for cell growth and hypertrophy.

Additional support comes from studies in which antioxidants prevent glomerular and renal hypertrophy, albuminuria, glomerular expression of TGF-β1 and extracellular matrix, and PKC activation in experimental diabetes (64,65,67–71). Reddi *et al.* (72) showed that a selenium-deficient diet caused an increase in albuminuria, glomerular sclerosis, and plasma glucose levels in both normal and diabetic rats; that TGF-β1 is a pro-oxidant; and that selenium deficiency increases oxidative stress *via* this growth factor.

Recent studies have suggested that loss of podocytes is an early feature of diabetic nephropathy and predicts a progressive course. Susztak *et al.* (73) showed an important role of apoptosis in this loss in models of type 1 and type 2 diabetes in mice. In *in vitro* studies, high glucose stimulated enhanced intracellular generation of oxidants in which both the NAD(P)H oxidase and mitochondrial pathways were involved. There was activation of p38 MAPK and caspase 3. Inhibition of NAD(P)H oxidase prevented apoptosis and reduced podocyte depletion, urinary albumin excretion, and mesangial matrix expansion.

Kang *et al.* (74) demonstrated that high glucose induces apoptosis in mesangial cells by an oxidant-dependent mechanism. The signaling cascade that is activated by glucose-induced oxidant stress included the heterodimeric redox-sensitive transcription factor NF-κB, which exhibited an upregulation in p65/c-Rel binding activity and suppressed binding activity of the p50 dimer. Perturbations in the expression and phosphorylation of the Bcl-2 family were coupled with the release of cytochrome *c* from mitochondria and caspase activation. The authors suggested that this may be a mechanism that accounts for the loss of resident glomerular cells that is observed in late diabetic nephropathy. The relation between oxidant stress and various pathways that have been implicated in diabetic nephropathy is summarized in Figure 1.

Role of Oxidants and Iron in Progressive Kidney Disease

The severity of tubulointerstitial injury is a major determinant of the degree and rate of progression of renal failure. There has been increasing interest in the possible link between exces-

sive protein trafficking through the glomerulus and progressive renal tubular interstitial inflammation that leads to chronic renal failure. In this section we first explore the link between proteinuria, oxidative stress, and activation of the pathways of interstitial inflammation. The role of inflammatory cells in progressive kidney disease has been reviewed (75). A candidate pathway for chemokine induction due to enhanced protein update is NF-$\kappa\beta$. Morigi *et al.* have shown that human proximal tubular cells incubated with human albumin (1 to 30 mg/ml) and IgG lead to a significant and rapid increase in H_2O_2 and activation of NF-$\kappa\beta$. Inhibitors of protein kinase C significantly prevented H_2O_2 production and consequent NF-$\kappa\beta$ activation (76) (Figure 2; Table 4).

A number of monocyte-specific cytokines have been described. MCP-1 has been identified as a product of a gene that belongs to the small, inducible cytokine family that is known in the murine system as the JE gene. IL-8 is a key proinflammatory chemokine responsible for recruiting and activating neutrophils, T cells, and monocytes. It has been shown that albumin is a strong stimulus for H_2O_2 production, which leads to activation of NF-κB–dependent pathways, resulting in increased expression of MCP-1 and IL-8, which are important in the inflammatory response (76,77). In addition, Gwinner *et al.* (78) showed in an *in vivo* model that hyperlipidemia leads to increased generation of oxidants through xanthine oxidase, resulting in increased expression of MCP-1 and vascular cellular adhesion molecule-1 and macrophage infiltration in the tubulointerstitial region.

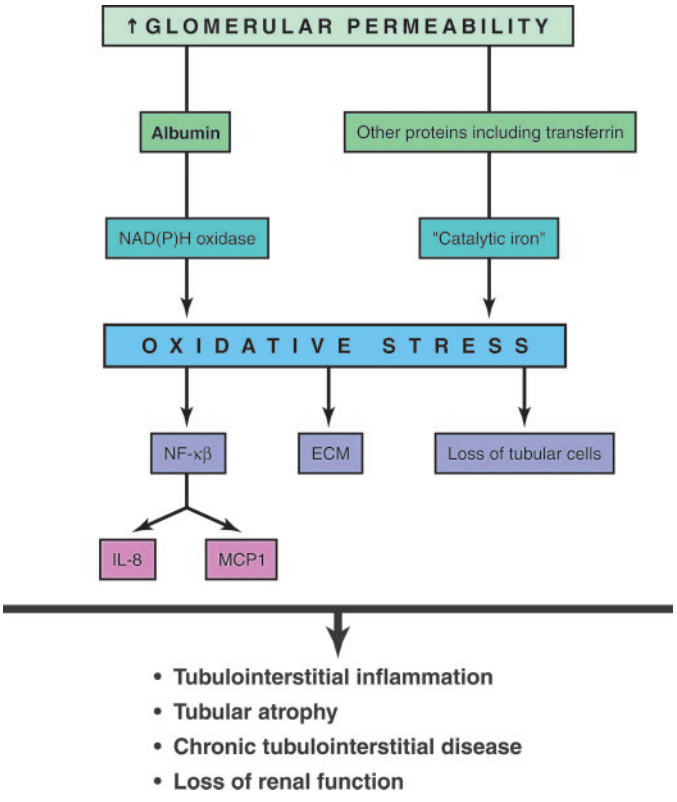

Figure 2. Relation of proteinuria to oxidant stress and tubulointerstitial injury. Illustration by Josa Gramling—Gramling Medical Illustration.

Tubular epithelial-to-mesangial transition (EMT) is a process in which renal tubular cells lose their epithelial phenotype and acquire new characteristic features of mesenchyme. There is growing evidence to implicate this process as a major pathway that leads to generation of interstitial myofibroblasts in diseased kidney (79). Rhyu *et al.* (80) showed that ROS mediate TGF-β1–induced EMT in renal tubular epithelial cells directly through activation of MAPK and indirectly through ERK-directed Smad 2 phosphorylation and suggested that antioxidants and MAPK inhibitors may prevent EMT through both MAPK and Smad pathways and subsequent tubulointerstitial fibrosis.

The evidence for the role of oxidants in progressive kidney disease has been reviewed and consists of the demonstration of the enhanced production of oxidants, evidence that oxidants induce similar morphologic and functional changes as seen in progressive kidney disease, and the beneficial effects of antioxidants (81). In this section, we emphasize the role of iron because of the possibility of using iron chelators to prevent progression. The data that support the role of iron in models of progressive renal disease consist of demonstration of increased iron in the kidney; enhanced oxidant generation, which provides a mechanism by which iron can be mobilized; and the beneficial effect of iron-deficient diets and iron chelators. Rats with proteinuria have increased iron content in proximal tubular cells, and iron accumulation was the only independent predictor of both functional and structural damage (82). Similarly, it has been shown that there is a substantial iron accumulation associated with increased cortical malondialdehyde in proximal tubular cells in the remnant kidney, suggesting ROS generation. The sources of increased iron in the kidney have not been well delineated, but Alfrey and colleagues (83,84) suggested that urinary transferrin provides a potential source of iron.

For iron to be important in causing renal injury, it is important also to demonstrate increased generation of oxidants. Oxidants then would play a role in mobilizing iron as well as interacting with the mobilized iron to generate highly reactive metabolites. Nath and colleagues (85,86) carried out a series of studies that have provided compelling evidence for the role of oxidants in progressive renal disease. Increased rates of oxygen consumption, which occur in surviving nephrons, are linked to ammoniagenesis and increased generation of ROM, both of which have been incriminated in progressive renal injury. It has been shown also that increased oxidant stress enhances ammoniagenesis, compromises renal function, and induces tubulointerstitial injury (85).

Several studies have demonstrated that an iron-deficient diet or iron chelators prevent the development of tubulointerstitial disease and renal functional deterioration in nephrotoxic serum nephritis (83,87). Remuzzi *et al.* (88) showed that rats that were fed an iron-deficient diet had a significant reduction in proteinuria and developed less glomerulosclerosis. An iron chelator significantly reduced iron accumulation and tubular damage in

Table 4. Oxidative stress and iron in progressive kidney disease[a]

Oxidative stress in progression of kidney injury (81)
generation of ROS
oxidative stress induces similar functional and morphologic changes
antioxidants protect against progression
albumin enhances production of oxidants, which lead to increased expression of IL-8 and MCP-1, known to play a role in tubulointerstitial inflammation (76,77)
Role of iron in progressive renal disease
iron is increased in the kidneys of animal models of progression (82,89)
progression of renal failure is prevented by iron-deficient diet (83,87), iron chelator
Human studies
increased iron content in kidney (112) and increased urinary catalytic iron
AOPP predict bad prognosis in IGA nephropathy (111)
metal chelator (EDTA) halts progression (113)

[a]AOPP, advanced oxidation protein products; MCP-1, monocyte chemoattractant protein-1.

the rat remnant kidney, a model for progressive renal disease (89).

Human Studies

A sufficient body of *in vitro* and *in vivo* information exists to postulate that oxidants seem to be important mediators in glomerular pathophysiology and progressive kidney disease. Although the collective information on the role of oxidants and iron that is derived from models of glomerular disease as well as progressive renal failure is impressive, there is little information on the potential role of these mechanisms in human disease. There are many differences between animal models of glomerular disease and glomerular disease in humans. For example, the animal model of minimal-change disease is a toxic model, whereas the mechanism of minimal-change disease in humans is not known. Similarly, the anti-Fx1A antibody that is used for the animal model of membranous nephropathy has been difficult to demonstrate in human membranous nephropathy. Indeed, the lessons from animal models of acute kidney injury have been disappointing when attempting to translate to human disease. We summarize the limited information from human studies that lend support that the mechanisms that are observed in animal models seem to be applicable to human disease.

Diabetic Nephropathy

Urinary Markers of Oxidative Stress. Several studies in humans have documented the presence of oxidative markers in the urine of patients with diabetes and correlate this with diabetic complications that include proteinuria. 8-Oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) is a product of oxidative DNA damage (90) that is known to be a sensitive marker of oxidative DNA damage and of oxidative stress *in vivo* (91). Several reports described an increase in the 8-oxodG content in the urine of patients with type 1 (insulin-dependent) and type 2 (non–insulin-dependent) diabetes (92–94), with the levels being significantly higher in patients with albuminuria (Figure 3B) or with other diabetic complications (95). Hinokio *et al*. (96), in a prospective, longitudinal study over 5 yr, found a significant progression of diabetic nephropathy in patients with a higher excretion of 8-oxodG in urine compared with patients with a moderate or lower excretion of 8-oxodG (Figure 3A). The multivariate logistic regression analysis suggested that urinary 8-oxodG is the strongest predictor of nephropathy among several known risk factors.

In a recent study, Monnier *et al.* (97) showed high levels of F2 isoprostane 8-iso prostaglandin $F_{2\alpha}$ (8-iso $PGF_{2\alpha}$), which is a widely recognized marker of oxidative stress in patients with diabetes. In this context, it is relevant that insulin decreases NAD(P)H oxidase activity (92).

Catalytic Iron. *In vivo*, most of the iron is bound to heme or nonheme protein and does not catalyze directly the generation of hydroxyl radicals or a similar oxidant (11). It has been shown that glycation of proteins leads to a substantial increase in the affinity for transition metals such as iron and copper (98). These glycochelates have the ability to participate in free-radical reactions. Iron chelators have been shown to improve coronary artery response to physiologic stimuli and blood flow in diabetes (99). It is interesting that recent studies have demonstrated that non–transferrin-bound iron levels frequently are increased in diabetes and have been implicated in a few studies with the vascular complications of diabetes (100).

The bleomycin-detectable iron assay is based on the observation that the antitumor antibiotic bleomycin, in the presence of iron salt and a suitable reducing agent, binds to and degrades DNA with the formation of a product that reacts with thiobarbituric acid to form a chromogen. The assay detects iron complexes that are capable of catalyzing free-radical reactions in biologic samples (11,101,102). In preliminary studies, we compared catalytic iron in individuals who had no renal disease or diabetes with patients who had diabetes (Figure 3C). Our data demonstrate that patients with overt diabetes have a marked increase in urinary catalytic iron. Similarly, patients with microalbuminuria have a marked and highly significant increase in urinary catalytic iron, indicating that urinary catalytic iron is not merely a reflection of albuminuria. Finally,

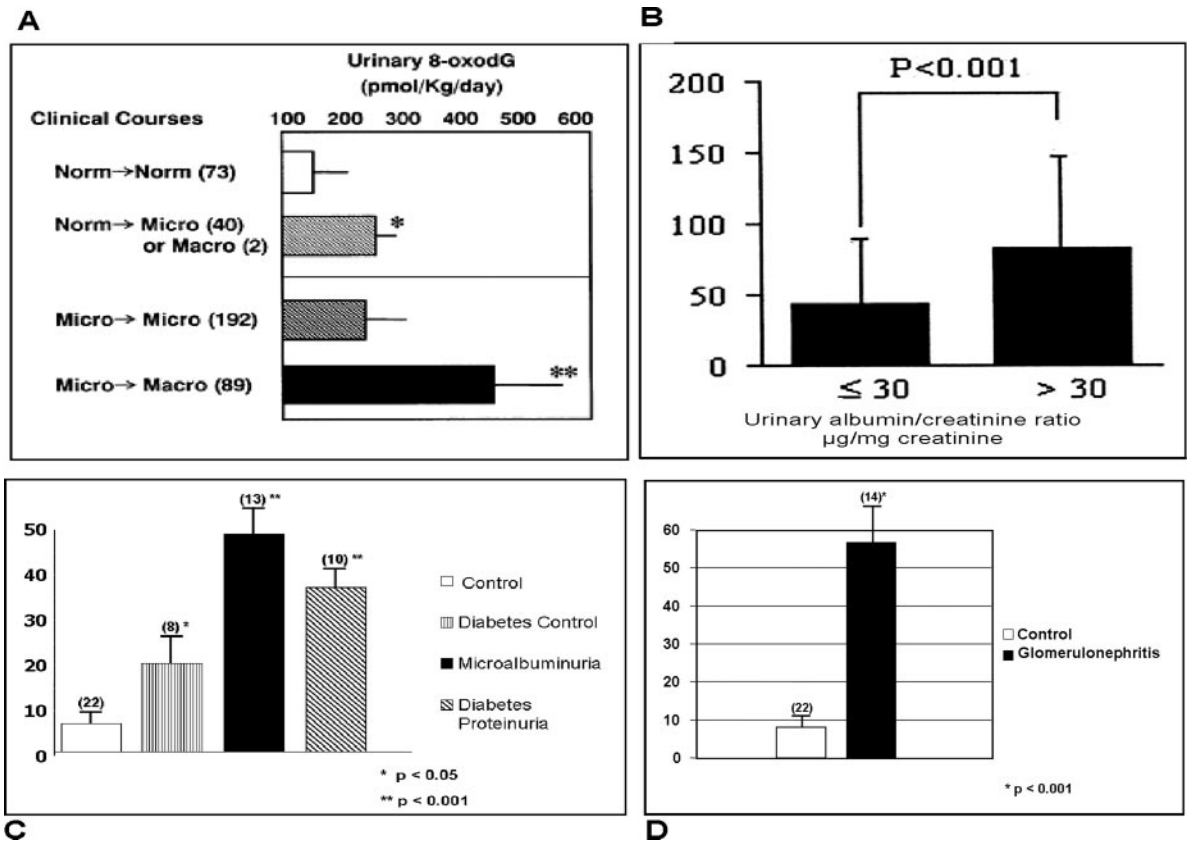

Figure 3. Urinary markers of increased oxidative stress in patients with diabetic nephropathy. (A) The contents of 8-oxo-7,8-dihydro-2′-deoxyguanosine (8-oxodG) in urine in patients who had type 2 diabetes with or without the progression of diabetic nephropathy (96). (B) The relationship between urinary 8-hydroxy-2′-deoxyguanosine (U8-OHdG) and microalbuminuria. Adapted from reference (95), with permission from The American Diabetes Association. Copyright © 2003 American Diabetes Association. (C) Urinary catalytic iron in patients with diabetic nephropathy. (D) Urinary catalytic iron in patients with biopsy-proven glomerulonephritis.

some patients in the diabetic control group who did not have microalbuminuria had high catalytic iron, leading us to postulate that urinary catalytic iron precedes the onset of microalbuminuria and may predict patients who are at risk for diabetic nephropathy.

Additional support for oxidants in diabetic nephropathy comes from the observation that diabetic nodular lesions in humans stained positive for malondialdehyde, an index of lipid peroxidation (103,104). In addition, Takebayashi *et al.* (104) showed that spironolactone treatment in patients with diabetic nephropathy caused a signification reduction in 8-iso-PGF2α accompanied by a reduction in MCP-1 and urinary albumin.

Other Forms of Progressive Kidney Disease

Evidence exists for the presence of increased oxidative stress in CKD (105–109). However, there is limited correlative and cause–effect information about the role of oxidants in progressive kidney disease. Kuo *et al.* (110) reported that plasma and urinary malondialdehyde, end products of lipid peroxidation, were increased significantly in patients with FSGS and concluded that oxidative stress occurs early and may play an important role in the pathogenesis of glomerular sclerosis. It has been suggested that advanced oxidation protein products (AOPP) may be important predictors of prognosis in IgA nephropathy. In a multivariate analysis, the most potent independent risk factors for poor renal outcome were proteinuria, hypertension, and AOPP plasma levels. Descamps-Latscha *et al.* (111) suggested that AOPP may serve as a surrogate marker for a bad prognosis and as a marker to evaluate the effectiveness of therapeutic modalities.

Nankivell *et al.* (112) reported increased iron content in patients with CKD. Using the urinary catalytic iron assay described before, we showed a marked increase in patients with biopsy-proven glomerulonephritis (Figure 3D). There is at least one study in the literature in which the effect of a metal chelator on progressive kidney disease has been examined. Lin *et al.* (113) showed that chelation therapy with EDTA in patients with chronic renal insufficiency results in reduced rate of decline in the GFR. The authors attributed the beneficial effect to the chelation of lead, which also participates in the Fenton reaction. However, given the affinity constants for iron and lead, the large experimental evidence for the role of iron in kidney disease, and the demonstrated efficacy of EDTA in enhancing excretion of urinary iron, we believe that the beneficial effects are more likely to be explained by chelation of iron rather than of lead (114).

Conclusion

In this review, we did not attempt to cover two important areas that are related to oxidants and are relevant to CKD. The first area relates to the role of reactive nitrogen species in kidney disease and the interaction between oxidants and reactive nitrogen species. The second area is the role of oxidants in hypertension. The reader is referred to several reviews on these subjects (115–123).

The conflicting data with vitamin E sometimes is cited as evidence against the role of oxidants in a disease process. It should be remembered that, other than usual issues with dosage and preparation, vitamin E's major effect is to prevent lipid peroxidation. Oxidants as described above effect many signaling and cellular processes that are unrelated to lipid peroxidation, which is considered a late event. Therefore, any conclusions that are derived from the vitamin E studies by and large should be restricted to the role of lipid peroxidation. An appropriate strategy to examine the role of oxidants is to block more proximal pathways, which have been linked to the pathophysiology of the disease process.

Several points from animal and human studies related to halting progression with a metal chelator are worth noting. The observation that reduction in proteinuria (provided that it is not attributable to a fall in GFR) results in slowing progression of kidney disease has been reasonably well established only with ACE inhibitors and ARB. This is in keeping with the data that albumin itself seems to have significant effects on tubular cells, including enhanced generation of oxidants and activation of the inflammatory response. In addition, as noted previously, other proteins, including iron-carrying proteins or complement components, also may have a detrimental effect. However, it is conceivable for a therapeutic agent to preserve the tubulointerstitial region by abolishing the consequences of proteinuria or having a direct protective effect on the tubules. Recent studies indicate that tubular changes represent more than just the aftermath of diabetic nephropathy, and functional and structural changes in the tubules may be a key to the development and progression of kidney dysfunction in diabetes. As an example, tubular hypertrophy is apparent after only a few days of hyperglycemia. Therefore, alterations in the kidney tubule may precede or at least accompany the pathognomonic changes in the renal glomerulus and the onset of albuminuria (124).

In nondiabetic CKD, both animal and human studies highlight the possibility of a beneficial effect without reduction in proteinuria. In studies by Alfrey and colleagues (83,87), an iron-deficient diet or iron chelator provided both functional and histologic protection against progression in a model of nephrotoxic serum without affecting proteinuria. Similarly, in the study by Lin *et al.* (113) described previously, EDTA provided protection against progression without reducing urinary protein. Therefore, clinical studies that target halting progression not only should focus on short-term studies on proteinuria but also would have to be of sufficient duration to evaluate the effect on renal function.

Disclosures

None.

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