Reactive Oxygen Species as Glomerular Autacoids

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ABSTRACT

There is considerable evidence suggesting that reactive oxygen species (ROS; superoxide anion, hydrogen peroxide, hydroxyl radical, hypochlorous acid) are implicated in the pathogenesis of toxic, ischemic, and immunologically mediated glomerular injury. The capacity of glomerular cells, especially mesangial cells, to generate ROS in response to several stimuli suggests that these autacoids may play a role in models of glomerular injury that are independent of infiltrating polymorphonuclear leukocytes and monocytes. The mechanisms whereby ROS formation results in morphologic lesions and in modifications of glomerular permeability, blood flow, and filtration rate have been inferred from in vitro studies. They involve direct and indirect injury to resident cells (mesangiolysis) and glomerular basement membrane (in concert with metalloproteases) and alteration of both the release and binding of vasoactive substances, such as bioactive lipids (e.g., prostaglandin E2, prostacyclin, thromboxane), cytokines (e.g., tumor necrosis factor alpha), and possibly endothelium-derived relaxing factor. The importance of such processes appears to be modulated by the intrinsic antioxidant defenses of the glomeruli. Further studies are needed to address the role of ROS in human glomerular diseases.

Key Words: Reactive oxygen species, glomerular cells, antioxidant enzymes, eicosanoids, cytokines, experimental glomerulonephritis

In the normally metabolizing aerobic cell, the molecule of oxygen is reduced to water with the addition of four electrons. Nevertheless, partial reduction can occur with the formation of highly reactive intermediates (reviewed in references 1-6). When oxygen undergoes the addition of only a single electron, it is converted to the superoxide anion (O$_2^-$) that can act either as an oxidant or a reductant. By definition, O$_2^-$ is a free radical because it contains one unpaired electron in its molecular orbital. Acceptance of the second electron results in the generation of hydrogen peroxide (H$_2$O$_2$), which is not a free radical but which is a relative stable oxidant. In combination with myeloperoxidase, H$_2$O$_2$ can oxidize halides such as Cl$^-$ to generate hypohalous acids (i.e., hypochlorous acid: HOCl), oxidants that in turn participate in the generation of another group of oxidants, the chloramines. Alternatively, H$_2$O$_2$ can oxidize a trace metal that has been reduced by O$_2^-$ to form hydroxyl radical (OH$^-$). OH$^-$ is an extremely powerful oxidant. All of these reactive intermediates are referred to collectively as reactive oxygen species (ROS). Because ROS are continually being formed in small amounts by normal processes of metabolism, intracellular enzymes exist that eliminate most of them. Superoxide dismutase (SOD) enzymes in the mitochondrial matrix and in the cytoplasm catalyze the dismutation of O$_2^-$ to form H$_2$O$_2$. Catalase in peroxisomes and glutathione peroxidase in the cytoplasm are capable of subsequently degrading H$_2$O$_2$ to water. A variety of nonenzymatic antioxidants including α-tocopherol, ascorbate, cysteine, and ceruloplasmin are also normally present in cells and extracellular fluid.

Occasionally, cellular production of ROS exceeds the capacity of the antioxidant enzymes that detoxify them. ROS excess results in destructive oxidation reactions with cell components including proteins, membrane lipids, and nucleic acids. There is considerable evidence suggesting that such an imbalance between the generation and degradation of ROS is implicated in the pathogenesis of toxic, ischemic, and immunologically mediated glomerular injury. This review will focus on the potential cellular sources of ROS and on the mechanisms of ROS toxicity within the glomerulus. Emphasis will be placed on the interactions between ROS and other inflammatory mediators including eicosanoids, cytokines, and reactive nitrogen intermediates.

EVIDENCE FOR A ROLE OF ROS IN GLOMERULAR PATHOPHYSIOLOGY

To demonstrate a role of ROS in a particular type of tissue injury, evidence should be presented that (1) ROS are detectable locally, (2) the chemical generation of ROS produces similar lesions, and (3) compounds able to remove ROS protect from the injury.
This evidence has been obtained from various in vivo and ex vivo studies.

Abnormal Production of ROS Within the Glomerulus

Recent studies have been designed to analyze the functional characteristics of glomerular macrophages obtained from isolated glomeruli by short-term culture (7,8). They demonstrated that, compared with blood monocytes, these cells produce larger quantities of O$_2^-$, H$_2$O$_2$, and OH$^-$ in models of acute proliferative glomerulonephritis. To confirm the generation of H$_2$O$_2$ within the glomerulus, the method employed by Guidet and Shah (9) could be applied as well because their method (aminotriazole-mediated inhibition of catalase) provides a measure of in vivo changes of H$_2$O$_2$ generation.

Local Generation of ROS Produces Glomerular Lesions

Numerous studies have shown that the infusion of a chemical or cellular source of ROS into the renal artery induces glomerular dysfunctions similar to those observed in glomerulonephritis, including injury of endothelial and mesangial cells, reduction of GFR, and alteration of glomerular permeability to proteins, resulting in proteinuria. For instance, Stratta et al. (10) demonstrated that H$_2$O$_2$ infusion into the renal artery induces mesangiolysis and endothelial detachment. When myeloperoxidase and H$_2$O$_2$ are successively infused into kidneys, hypohalous acid is generated and injury to the endothelium is associated with proteinuria (11,12). This is accompanied by an increase in the fractional clearance of neutral dextran (13). Finally, studies analyzing the role of H$_2$O$_2$ either generated by polymorphonuclear leukocytes upon phorbol myristate acetate challenge (14) or directly infused into the renal artery (15) indicated that ROS promotes a fall in GFR and ultrafiltration coefficient and an increase in renal arterial resistances.

Agents Removing ROS Provide Protection Against Glomerular Lesions

The question of ROS involvement in the glomerular inflammatory reaction has led many investigators to attempt to block ROS toxicity by interfering with either their generation or their metabolism. They have demonstrated that, with few exceptions, SOD does not provide protection against glomerular functional impairment after local deposition of immune complexes. By contrast, SOD administration reduces glomerular morphologic changes and proteinuria in a model of minimal-change disease obtained by a single i.v. injection of the aminonucleoside of puro-mycin (16,17). The administration of catalase reduces the degree of glomerular injury, as assessed by the reduction of proteinuria, in the heterologous phase of antilglomerular basement membrane (anti-GBM)-Induced glomerulonephritis (18). In two models of membranous nephropathy induced either by a single i.v. injection of anti-FxI A antibody (passive Heymann nephritis) (19) or by repeated i.v. injections of cationic bovine y-globulin to preimmunized rats (20), SOD and catalase do not affect proteinuria. In contrast, the administration of OH$^-$ scavengers, such as dimethylsulfoxide, and dimethylthiourea, or of desferrioxamine, an iron chelator that prevents OH$^-$ generation, results in significant decrements of proteinuria, without modification of glomerular immunoglobulin and complement deposition.

Several lines of evidence have established that polymorphonuclear leukocytes and monocytes/macrophages are potential sources of ROS within the glomerulus. Rehan et al. (18) have reported that the ROS-dependent proteinuria in the heterologous phase of anti-GBM-induced glomerulonephritis is greatly diminished when animals are neutrophil depleted. The same authors have shown that, conversely, the intravascular activation of neutrophils by the infusion of phorbol myristate acetate (PMA) (21) or cobra venom factor (22) into the renal artery causes ROS-dependent glomerular injury and proteinuria. However, because blood-borne cells do not contribute to the pathogenesis of proteinuria in the models of membranous nephropathy (19,20), the question arises as to whether glomerular cells themselves represent a second source of ROS within the glomerulus.

EVIDENCE FOR THE CAPACITY OF MESANGIAL CELLS TO GENERATE ROS

Using luminol-amplified chemiluminescence, Shah (23) first demonstrated that glomeruli isolated from rat kidneys generate ROS in response to the addition of PMA. Subsequent studies have shown that this generation, which does not depend on the presence of bone marrow-derived phagocytes (2), is amplified by the addition of neutral proteases (trypsin and chymotrypsin) (24) and is limited by the addition of agents increasing cAMP accumulation (25). More recently, using aminotriazole-mediated inhibition of catalase, Guidet and Shah (9) have confirmed that glomeruli generate H$_2$O$_2$ in vitro under normal conditions. Endothelial, mesangial, and epithelial cells could contribute to ROS production by isolated glomeruli. Studies with glomerular cells in culture have indicated that at least mesangial cells from both murine (26) and human (27) origins have the potential to generate ROS. The magnitude of this production is greater than that exhibited by epithelial
cells from the proximal tubule, the cortical collecting duct, and the papillary collecting duct (28). Both O$_2^-$ and H$_2$O$_2$ are released upon the interaction of mesangial cells with opsonized zymosan (26), immunoglobulin G and immunoglobulin A immune complexes (29,30), complement membrane attack complex (31), PMA (27), A23187 calcium ionophore, platelet-activating factor (PAF) (32), and cytokines including interleukin 1$\alpha$ and tumor necrosis factor alpha (TNF-$_\alpha$) (27). Arachidonic acid release from its phospholipid stores is induced by the exposure of mesangial cells to these agents and could be involved in the biochemical events responsible for ROS generation. For example, studies from our laboratory have documented that the pretreatment of mesangial cells with dexamethasone induces a reduction of H$_2$O$_2$ generation through a receptor-mediated mechanism. This is mediated in part by a decrease in arachidonic acid availability, because the addition of exogenous arachidonic acid reduces the inhibitory effect of the glucocorticoid (32). Arachidonic acid release induces ROS generation by bloodborne cells as well (33). However, the mechanism by which arachidonic acid promotes the activation of these cells remains unclear. In addition to glucocorticoids, agents increasing cAMP accumulation (histamine, adenosine, forskolin) reduce ROS generation by mesangial cells (34,35).

Finally, the capacity of both glomerular epithelial and endothelial cells to generate ROS upon exposure to the aminonucleoside of puromycin, and to TNF-$_\alpha$, respectively, has been demonstrated in two preliminary studies (36,37). These data, in combination with the in vitro studies reported above, provide additional support for ROS-mediated glomerular damage in the mesangial matrix (34.35).

EVIDENCE FOR THE CAPACITY OF GLOMERULAR CELLS TO EXPRESS ENZYMATIC DEFENSES AGAINST ROS

The ROS-induced glomerular lesions are due to an imbalance between ROS generation and local expression of antioxidant enzymes. Thus, it was important to determine the levels of these enzymes in glomerular cells. Steinert et al. and Yang et al. (38,39) have first reported that glomeruli isolated from guinea pig kidneys show the activities of both copper-zinc and manganese forms of SOD, catalase, and glutathione peroxidase. Culture of these glomerular cells induces an increase in SOD activity, a decrease in catalase activity, and variable glutathione peroxidase activity. Compared with those of proximal tubules, the activities of SOD and glutathione peroxidase in glomeruli are weak. These dissimilarities have been confirmed in immunohistochemical studies from the same laboratory (40). They have documented no immuno-staining of glomeruli with anti-SOD and anti-catalase antibodies, whereas tubules stained. The possibility that the glomerular expression of antioxidant enzymes may be modulated in vivo has also been considered. Yoshioka et al. and Kawamura et al. (41,42) have demonstrated that the activities of SOD, catalase, and glutathione peroxidase increase more than twofold in glomeruli 6 days after a 30-min episode of complete renal ischemia or after repeated injections of methylprednisolone. Clearly, the enhanced activities of antioxidant enzymes improve the effective defense system of glomerular cells against ROS-mediated injuries because kidneys demonstrate resistance against H$_2$O$_2$ and the aminonucleoside of puromycin-induced proteinuria under these conditions. Thus, it is tempting to speculate that the mechanisms for the therapeutic effect of glucocorticoid administration in experimental glomerulonephritis include both reduction of ROS generation and enhancement of endogenous glomerular antioxidant enzyme activities. Finally, the availability of transgenic mice with overexpression of the copper-zinc form of SOD has enabled recent studies of its function in the kidney. Preliminary results (43) indicate an attenuation of morphologic and functional damage after 25 min of renal ischemia in transgenic mice as compared with control mice.

POTENTIAL MECHANISMS OF ROS-MEDIATED GLOMERULAR INJURY

A series of in vitro studies has shown the ability of ROS to affect the structure and the function of glomerular cells and GBM. Shah et al. (44) demonstrated that PMA-stimulated neutrophils cause a significant degradation of GBM, as evidenced by the increased release of hydroxyproline. Catalase, scavengers of hypochlorous acid, and inhibitors of metalloproteinases markedly reduce the degradation, suggesting the activation of a latent metalloproteinase by a product of the myeloperoxidase-H$_2$O$_2$-Cl$^-$ system. ROS-induced GBM Injury may also be mediated through the destruction of proteinase inhibitors or by the potentiation of proteinase action (6). The cellular responses to ROS include cytolysis, contraction, proliferation, and production of cell matrix—events that require the generation of intermediary mediators such as eicosanoids, PAF, cytokines, or reactive nitrogen intermediates.

EFFECTS OF ROS ON THE PRODUCTION OF EICOSANOIDS AND PAF

Isolated rat glomeruli have been shown to generate prostaglandin E$_2$ (PGE$_2$), PGF$_{2\alpha}$, and, to a lesser extent, 6-keto-PGF$_{1\alpha}$ and thromboxane B$_2$, the stable
This increase is due to the generation of H2O2 because we and others have reported on the biophase action of ROS upon eicosanoid production. We first observed that the addition of enzymatically generated ROS to incubation media causes a dose-dependent increase in the glomerular production of PGE2, PGF2α, 6-keto-PGF1α, and thromboxane B2 (46). This increase is due to the generation of H2O2 because (1) it is inhibited by catalase, slightly stimulated by SOD, and unaffected by O2* scavengers, and (2) it is obtained after the addition of H2O2 as well (1 to 100 μM). Additional experiments with [14C]arachidonic acid-labeled cells suggested that H2O2 increases prostaglandin synthesis by enhancing arachidonic acid availability. The mechanism may involve a Ca2+ flux across the plasma membrane, with activation of phospholipase A2 activity. The main consequence of ROS-induced prostaglandin synthesis is an accumulation of cAMP within glomerular cells (47,48). By contrast, higher levels of H2O2 suppress PGE2 generation when glomerular cells are stimulated with the calcium ionophore A23187 or with arachidonic acid (49). Both the increase and decrease of eicosanoid synthesis might modulate glomerular functions and the inflammatory process in glomerular diseases.

Isolated rat glomeruli and cultured mesangial cells have been shown to generate PAF in addition to eicosanoids (50). Preliminary studies indicate that ROS, and especially H2O2, enhance this generation (51). It is plausible that ROS-induced PAF synthesis participates in the proliferation of mesangial cells (51) and in the decrease of the ultrafiltration coefficient (52) that characterize certain forms of glomerular inflammatory disease.

EFFECTS OF ROS ON THE FORMATION AND ACTIVITY OF TNF-α WITHIN THE GLOMERULUS

Several reports indicate that both ROS and TNF-α are produced within the glomerulus and can contribute to the pathology of immune complex glomerulonephritis. The most obvious demonstration is in the autologous phase of anti-GBM antibody-induced glomerulonephritis. In this model, glomerular macrophages are locally activated to produce significant quantities of ROS and cytokines, including TNF-α (53). In fact, ROS and TNF-α might be generated by resident glomerular cells, besides bloodborne cells. In support of this hypothesis is the observation that rat cultured mesangial cells express TNF-α mRNA and generate TNF-α molecules (54,55). Whatever its cellular source, this cytokine is not only secreted as a 17-kd soluble protein, but is also expressed as a cell surface-associated 26-kd molecule. Its synthesis starts with the elaboration of the 26-kd molecule, which is subsequently cleaved during processing to yield the mature 17-kd protein. The first evidence for interactions between ROS and TNF-α derives from studies by Chaudhri and Clark (56). They have demonstrated that the release of TNF-α from lipopolysaccharide (LPS)-activated macrophages is enhanced by H2O2 (10 to 50 μM). Conversely, several antioxidants as well as desferrioxamine cause a dose-dependent reduction of TNF-α release. To investigate the mechanisms by which ROS increase TNF-α release, we have extended these observations to the study of rat mesangial cells (55). We have demonstrated that desferrioxamine also dose dependently decreases TNF-α generation from these cells. Time-course studies led to the conclusion that there is first a delayed release of the cytokine and then an acceleration of its decay. After 2 h of incubation with LPS, the release of TNF-α from mesangial cells into the medium is reduced by desferrioxamine, whereas the expression of TNF-α increases significantly at the cell surface. One interpretation for the desferrioxamine effect might be that this drug prevents the generation of OH· and, hence, reduces the ROS-dependent activation of proteases required to clip the extracellular domain of the cell-associated TNF-α molecule. An alternative hypothesis could be that this drug impairs TNF-α synthesis at the level of gene transcription as well as at a posttranscriptional level.

When measured after incubation periods of longer than 4 h, the activity of TNF-α in the culture medium of LPS-activated mesangial cells appears to decline faster in the presence of desferrioxamine than under control conditions. Most likely, the mechanism by which this drug enhances the inactivation of TNF-α does not involve a modification of TNF-α cleavage by proteolytic enzymes but rather involves an increase of TNF-α binding to specific inhibitors. These TNF-α-binding proteins have been identified as truncated soluble forms of the transmembrane TNF-α receptor. Of interest, desferrioxamine has been recently shown to enhance the binding of radiolabeled TNF-α to both cell membrane TNF-α (57) and truncated soluble TNF-α receptors (58), possibly by reducing the oxidative damage of the cysteine-rich subdomain of the molecule. Thus, the higher rate of TNF-α inactivation observed in the presence of desferrioxamine may reflect its increased binding to soluble receptors. In glomeruli, the ROS-induced increase of TNF-α release may potentially promote a reduction in the GFR, coagulation, inflammatory response, and sclerosis (58).

EFFECTS OF ROS ON THE ACTIVITY OF NITRIC OXIDE WITHIN THE GLOMERULUS

There are recent reports that nitric oxide (NO) and its major end products nitrite (NO2−) and nitrate (NO3−) are synthesized within the glomerulus in-ex-
peripheral immune complex glomerulonephritis (59). Macrophages are the most likely source of these metabolites. However, besides macrophages, resident glomerular cells might contribute to their generation, because cultured bovine (60) and rat (61) mesangial cells have been demonstrated to generate L-arginine-derived NO and NO$^-$/NO$_2^-$ upon exposure to TNF-$\alpha$ or interleukin-1. NO formation has been evidenced indirectly by the determination of the accumulation of cGMP, which is inhibited by methylene blue and hemoglobin. NO$^-$/NO$_2^-$ production has been demonstrated spectrophotometrically with the Griess reagent. The half-life of NO is markedly shortened by a simultaneous production of O$_2^-$ by these cells. Indeed, the addition of SOD to the culture medium appears to augment the TNF-$\alpha$-induced effect (60). Given that NO-induced cGMP accumulation within the glomerulus causes relaxation and increased GFF, degradation of NO may represent another mechanism by which ROS affect local circulation.

CONCLUSION

Many in vivo and in vitro studies provide strong support for the concept that ROS generation may significantly contribute to glomerular injury in both leukocyte-dependent and leukocyte-independent models of glomerular disease. The mechanisms of ROS-mediated glomerular injury involve their interaction with different inflammatory mediators, including eicosanoids, PAF, cytokines, and reactive nitrogen intermediates. Future work should aim at confirming these observations in human diseases.

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