Invited Comment

Role of reactive oxygen species in glomerulonephritis

Wilfried Gwinner¹ and Hermann-Josef Gröne²

¹Department of Nephrology, Medical School, Hannover, and ²Department of Cellular and Molecular Pathology, German Cancer Research Center (DKFZ) Heidelberg, Germany

Introduction to reactive oxygen species and their pathophysiology

Small amounts of reactive oxygen species (ROS) are constantly produced in aerobic metabolism and have important roles in normal cell physiology e.g. signal transduction pathways. However, in pathophysiological conditions with increased levels of ROS, these molecules become relevant factors in the initiation and amplification of deleterious processes observed in inflammation, oncogenesis, and degenerative diseases [1,2].

ROS are products of the partial reduction of oxygen and can be generated by enzymatic and non-enzymatic reactions within cells and at the cell membrane [3,4]. Enzymes that generate ROS are termed oxidative enzymes (Table 1) [5–16]. Major ROS are depicted in Figure 1, which also shows the important reactions and catalysts involved. Hypochlorous acid produced by myeloperoxidase and hydroxyl radical (OH'), which evolves from non-enzymatic reactions dependent on the availability of the free metal ions Fe²⁺ or Cu⁺, are the most reactive of the ROS [17]. Several defence mechanisms exist to decrease the concentration of ROS. Superoxide dismutases (SODs) catalyse the dismutation of superoxide anion to hydrogen peroxide (H₂O₂), which is further degraded by glutathione peroxidases and catalase (Table 1) [18]. Besides these antioxidative enzymes, metal-binding proteins such as ferritin, transferrin, caeruloplasmin, and metallothionin are present to limit the generation of OH'. The antioxidant defence is further complemented by the vitamins A, E, and C, and by bilirubin which act as scavengers of ROS [3,4].

Thus the balance between oxidative and antioxidative enzymes and other antioxidative components determines the concentration of ROS and thereby, physiological or pathophysiological ROS-related cellular effects. However, this simplified scheme is complicated by the fact that oxidative and antioxidative enzymes are localized in specific extracellular and intracellular compartments (Table 1). In addition, the expression of oxidative and antioxidative enzymes varies between different organs and within different cell types of an organ. Microlocal concentrations of ROS can therefore differ appreciably in a single cell or organ compartment (e.g. glomerulus, tubule, and interstitium of the kidney) [20]. Based on this compartmentalization of ROS synthesis and degradation, it can be deduced that examination of ROS and the enzymes involved in their metabolism in samples of

Table 1. Cellular localization of oxidative and antioxidative enzymes [5–16]

<table>
<thead>
<tr>
<th>Enzyme</th>
<th>Localization</th>
</tr>
</thead>
<tbody>
<tr>
<td>Xanthine oxidoreductase</td>
<td>Cytoplasm, peroxisomes</td>
</tr>
<tr>
<td>NADPH–Oxidase</td>
<td>Cell membrane, granula of leukocytes, nuclear membrane</td>
</tr>
<tr>
<td>NADH–Oxidase</td>
<td>Cell membrane, microsomes, mitochondria</td>
</tr>
<tr>
<td>Aldehyde oxidase</td>
<td>Cytoplasm, mitochondrial, microsomes</td>
</tr>
<tr>
<td>Sulphite oxidase</td>
<td>Cytoplasm, mitochondrial, microsomes</td>
</tr>
<tr>
<td>P450 – System</td>
<td>Microsomes</td>
</tr>
<tr>
<td>Cu,Zn-SOD</td>
<td>Cytoplasm, mitochondrial, lysosomes</td>
</tr>
<tr>
<td>Mn-SOD</td>
<td>Mitochondria</td>
</tr>
<tr>
<td>Catalase</td>
<td>Peroxisomes, cytoplasm</td>
</tr>
<tr>
<td>Glutathione peroxidases</td>
<td>Mitochondria, cytoplasm, peroxisomes, nuclear membrane</td>
</tr>
</tbody>
</table>

Cu,Zn-SOD, copper/zinc-superoxide dismutase; Mn-SOD, manganese superoxide dismutase.

Correspondence and offprint requests to: Hermann-Josef Gröne MD, Department of Cellular and Molecular Pathology, German Cancer Research Centre (DKFZ) Heidelberg, Im Neuenheimer Feld 280, D-69120 Heidelberg, Germany.

© 2000 European Renal Association–European Dialysis and Transplant Association
plasma, urine or whole-organ homogenates can easily miss changes in the oxidative status that are confined to a specific target, e.g. podocytes in membranous glomerulonephritis (GN) or mesangial cells in anti-Thy 1 nephritis.

Locally increased concentrations of ROS may lead to tissue damage by several mechanisms. As a result of direct oxidative modification of cellular components, cell structure and function can be altered substantially. (i) Oxidation of lipids generates lipid radicals which can, in turn, initiate and self-sustain lipid oxidation (Figure 2) [21,22]. Thus, cell and basement membranes that depend on the integration of non-oxidized lipids to maintain their orderly architecture may be deranged, a process that could be important for glomerular proteinuria. (ii) Oxidative modification of protein residues can promote the loss of the scaffolding property of structural proteins, can inactivate enzymes, and finally can alter the degradation and clearance of these molecules [23,24]. (iii) Oxidation of purines, pyrimidines and of the attached ribose moiety can give rise to cross-linking or fragmentation of nucleic acids, leading to altered gene expression [19,25].

Besides this direct oxidative damage to cellular components, increased ROS may act as trans- and intracellular signals that activate or inactivate redox-sensitive protein kinases or phosphatases resulting in altered phosphorylation of receptors and transcription factors. The ensuing changes in transcription factor activities can lead to critical changes in the expression of cytokines, adhesion molecules, and proteins involved in proliferation and apoptosis, thereby affecting inflammation, cell-to-cell contact, and cell death [26–28].

**ROS in glomerular disease**

Animal experiments have provided ample evidence that ROS play a major role in many glomerular diseases (see Table 2). Most of this evidence has been obtained indirectly by the detection of products of lipid peroxidation in renal tissues and by the demonstration of protective effects of administered antioxidants. This short review will only focus on results of direct examination of ROS and the enzymes involved in ROS metabolism.

**Equivalents to minimal-change glomerulopathy (MCG)**

Treatment of rats with puromycin aminonucleoside (PAN) leads to temporary glomerular proteinuria with ultrastructural glomerular lesions that resemble those seen in human MCG. Minutes after PAN injection, H$_2$O$_2$ and OH$^-$ increased in glomeruli, presumably due to a direct effect of PAN on podocytes. One and 5 days later glomerular ROS levels were normal and rose again 9 days after nephrosis induction [29–31]. The oxidative enzyme(s) causal for increased ROS have not been identified yet [32]. Decreased activities of glomerular antioxidative enzymes may be, at least partly, responsible for increased ROS levels [29,33,34]. Another model of human MCG is adriamycin nephrosis in rats, which is characterized by continuous proteinuria after induction of glomerular disease. In this model, xanthine oxidoreductase (XDH/XO) may be involved as a source of ROS as suggested by increased xanthine oxidase activity in renal homogenates of nephrotic animals and by a significant reduction of proteinuria after inhibition of the enzyme [35]. Renal activities of antioxidant enzymes remained essentially unchanged in adriamycin nephrosis [36–40].

**Equivalent to mesangioproliferative GN**

Anti-Thy 1 nephritis in rats is an immune complex GN with pronounced mesangial cell proliferation after an initial phase of mesangial cell death and lysis. As early as 2 h after administration of anti-Thy 1 immunoglobulin, glomerular ROS (predominantly H$_2$O$_2$) were increased, reaching a peak after 24 h [41]. In parallel with these biochemical results, histochemical analyses located increased formation of H$_2$O$_2$ in the leukocytes that had infiltrated the glomeruli [42]. As glomerular NAD(P)H oxidase activities were elevated at the same time, activation of leukocyte NADPH oxidase may be the major cause of increased glomerular ROS in this model. Glomerular activities of SODs, catalase, and glutathione peroxidases were decreased, which may
have further contributed to increased glomerular ROS [41].

In contrast to these observations in the acute phase of nephritis, ROS did not seem to participate in the glomerular scarring and tubulointerstitial fibrosis in a chronic model of anti-Thy 1 nephritis. However, this changed dramatically in the presence of concomitant hyperlipidaemia, which is regarded as a progression factor in renal disease in patients and experimental animals. In the animals with chronic nephritis, hyperlipidaemia induced a rise in ROS in glomeruli and cortical tubulointerstitium. This increase in ROS could be associated with a higher incidence of glomerulosclerosis and mostly of tubular atrophy and interstitial fibrosis. XDH/XO apparently was the major oxidative enzyme responsible for increased ROS in glomeruli and tubulointerstitium, as demonstrated by increased xanthine oxidase activity and elevated XDH/XO mRNA and protein levels in renal tissues. With regard to the antioxidant defence, glomerular and tubulointerstitial antioxidative enzymes remained essentially unchanged [43]. Recent unpublished results of our group imply that XDH/XO may be critically involved in early processes of hyperlipidaemia-induced progression of renal disease as inhibition of xanthine oxidase prevented the early influx of monocytes into the tubulointerstitium in animals with hyperlipidaemia (Gwinner et al., unpublished data).

**Equivalent to membranous GN**

Active and passive Heymann nephritis (HN) is induced by a complement-dependent immune reaction with components of the renal brush border, including megalin and an associated protein RAP, and is regarded as an experimental equivalent to human membranous GN. In the passive variant of HN, increased H$_2$O$_2$ could be demonstrated in the glomerular basement membrane and glomerular epithelial cells by histochemistry. This was associated with the appearance of cytochrome b$_{558}$, a critical component of NADPH oxidase, in glomerular epithelial cells, with a maximum 7 days after induction of nephritis [44].

Another oxidative enzyme involved in passive HN is xanthine oxidase. Increased glomerular activity of this enzyme was observed as early as one day after induction of nephritis, together with an increase in glomerular $\cdot O_2^-$ levels. Inhibition of xanthine oxidase decreased glomerular $\cdot O_2^-$ and proteinuria in nephritic animals, thus confirming an important role of this oxidative enzyme [45]. In addition to increased generation of ROS, decreased degradation of ROS may contribute to glomerular oxidative injury in passive HN as antioxidative enzyme activities declined moderately in renal tissues in the course of nephritis [46].

**Equivalent to focal segmental necrotizing GN**

In antibasement membrane GN in rabbits, an increased synthesis of $\cdot O_2^-$, H$_2$O$_2$, and OH$^-$ has been observed in leukocytes isolated from diseased kidneys [47]. Similar results have been obtained in rats by histochemistry, demonstrating increased ROS generation in the leukocytes that had infiltrated the glomeruli [42]. Although leukocyte NADPH oxidase may be the likely source of ROS, no studies exist which systematically examine the relative contribution of NADPH oxidase, myeloperoxidase, and other potentially important oxidases of leukocytes in this model. With regard to the antioxidative enzymes, activities of SOD, catalase, and glutathione peroxidases decreased in renal tissues in the course of antibasement membrane GN in rats [46].

**Lessons from the animal studies and applications to human glomerular disease**

From experimental observations it is becoming increasingly evident that oxidative injury in glomerular disease may result primarily from the induction of specific oxidative enzymes in resident glomerular cells and in leukocytes infiltrating the kidney. In contrast to in vitro observations in cultured glomerular cells [48], glomerular antioxidative enzyme expression appears to be decreased rather than induced in response to increased ROS in most glomerular disease models, thus further contributing to the oxidative burden. However, data are still fragmentary with regard to the analysis of different ROS and their temporal profile during the course of experimental glomerular diseases. Ultrastructural localization of increased ROS synthesis and altered oxidative and antioxidative enzyme expression within the glomerulus has been incomplete. The mechanisms leading to the changes in oxidative and antioxidative enzyme expression are largely unknown. The effect of locally increased ROS on cellular signaling events including induction of redox-sensitive pathways and the ensuing expression of mediators of glomerular inflammation and fibrosis awaits further exploration in the in vivo models of glomerular disease. Such detailed analyses will be necessary to develop rational and specific therapeutic strategies.

Although experimental studies have shed some light on the potential mechanisms involved in oxidative glomerular injury, a crucial question is whether the same mechanisms are active in human GN. Hitherto, data are lacking to answer this question. Measurement of ROS in glomeruli of patients with GN has not been performed. The increased generation of $\cdot O_2^-$ in blood leukocytes of patients with pauci-immune-complex GN that are positive for antineutrophilic cytoplasmic antibodies, may be taken as indirect evidence that leukocytes in necrotizing glomeruli could cause injury by ROS [49]. Also, the oxidative enzymes involved in increased glomerular ROS generation have not been identified in human GN. With regard to antioxidative enzymes, immunohistochemical studies in different forms of GN indicated increased glomerular expression of SODs and catalase, predominantly in patients with IgA nephropathy and lupus nephritis [50]. However,
data are inconclusive. Whereas one study showed an increased expression of Cu,Zn-SOD in the mesangial area of severely affected glomeruli in patients with IgA nephropathy, other studies reported strongest expression of Cu,Zn-SOD and glutathione peroxidase predominantly in epithelial cells of glomeruli with minor disease involvement [51–53].

**Therapeutic modulation of oxidative injury in human GN**

Strategies to reduce or prevent oxidative glomerular injury may be realized in two different ways, by inhibition of increased ROS generation and by enhanced degradation of ROS and oxidatively modified cellular components.

**Inhibition of increased ROS generation**

Inhibition of increased ROS generation may be considered as a relatively specific approach but a prerequisite is that the responsible oxidative enzyme(s) or catalyst(s) have been identified. Clinically available inhibitors of xanthine oxidase include allopurinol, oxy-purinol, amflutizole [54], and tenidap [55]. Yet, their therapeutic efficacy has not been examined in human GN. Specific inhibitors of NAD(P)H oxidases are not currently available for clinical use. Inhibitors of angiotensin II converting enzyme (ACE inhibitors) and angiotensin II receptor antagonists may exert some inhibitory effects on NADPH oxidase as angiotensin II increases NADPH oxidase activity [56,57]. The therapeutic potential of certain phosphodiesterase inhibitors which may reduce the activation of NADPH oxidase in leukocytes [58] and glomerular cells [59] has not been examined in human GN.

**Enhanced degradation of ROS**

Several substances can facilitate the enhanced degradation of ROS and partly, of oxidatively modified components: (i) antioxidative enzymes or low-molecular mimics of antioxidative enzymes which catalyse the degradation of ROS, (ii) scavengers that combine with ROS and oxidatively modified components, thereby neutralizing their oxidative potential, and (iii) stimuli that increase the expression of intrinsic antioxidative enzymes.

(i) SOD has been used in preservation solutions in renal transplantation, resulting in improved long-term function of allografts [60]. In GN, the efficacy of antioxidative enzymes has not been evaluated. Arguments against therapy with antioxidative enzymes are the necessity of parenteral administration and the lack of intracellular uptake. Low-molecular mimics with SOD- and glutathione peroxidase-like activity are capable to penetrate cell membranes [61–63] and may be therefore an attractive alternative to be tested in human GN.

(ii) Potent scavengers of ROS do exist but some of them such as dimethylthiourea and dimethylsulphoxide are not suitable for human use. Likewise, chronic use of the metal chelator desferrioxamine would be limited by unwanted effects including anaemia. The vitamins C and E and the sulph-hydryl compound acetylcysteine have been proposed as ROS scavengers in human GN [64–66]. To date, only preliminary data are available that indicate a beneficial effect of vitamin E on proteinuria in focal segmental glomerulosclerosis [65] and of the antioxidant probucol on proteinuria in membranous GN [67]. Besides these well-defined ROS scavengers some clinically established drugs have shown scavenger properties such as captopril, carvedilol, and calcium antagonists [68–70]. However, it is unknown, whether this effect plays a significant role in the prevention of oxidative injury in GN.

(iii) Several stimuli have been reported to induce the expression of intrinsic cellular antioxidative enzymes. However, only a few of these stimuli may be of clinical value. For instance, the induction of manganese SOD by interleukin 1, tumour necrosis factor, and endotoxin [71,72] is associated with an unwanted increase in ROS generation. ACE inhibitors induce SOD, catalase, and glutathione peroxidase activities in renal tissues of rodents [73–75], and their therapeutic efficacy in human glomerular disease may be explained, in part, by this effect. An induction of glomerular antioxidative enzymes has been also shown after steroid administration to rats [33]; however, as steroids can induce the expression of XDH [76] the net effect on the glomerular oxidative–antioxidative balance remains undetermined. Administration of metal cofactors (selenium, manganese, copper, zinc) of anti-oxidative enzymes has been suggested in the therapy of renal disease, because lack of these factors may exacerbate oxidative injury [64]. It is unknown whether supplementation with these factors sufficiently increases antioxidative enzyme activities; the risk of intoxication has certainly to be considered.

With regard to therapies aiming at enhanced degradation of ROS some general concerns need to be raised. The coordinate decomposition of \( \cdot O_2^\cdot, H_2O_2, \) and OH’ may require the use of more than one antioxidative substance. Yet certain combinations of antioxidants have shown pro-oxidant effects [77]. The ROS scavenger vitamin E can turn into a pro-oxidant if vitamin C is not present in sufficiently high concentrations [22]. Another concern is whether the antioxidative substance is capable of reaching the compartment where increased concentrations of ROS are present. Lastly, reduction of ROS below a physiological range in certain cell organelles and compartments may disturb the oxidative metabolism necessary for coordinate physiological gene transcription and other cell functions. The rational therapy of oxidative injury in GN may therefore prove to be quite difficult and will only succeed when an integration of therapeutic antioxidant strategies is achieved into the complex interaction of intra- and extracellular antioxidative–oxidative mechanisms.
References


