Chapter 25
Oxidative Stress in Kidney Diseases

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Abbreviations

AKI	Acute kidney injury
CKD	Chronic kidney disease
CPB	Cardiopulmonary bypass
EMT	Epithelial-to-mesangial transition
EPO	Erythropoietin
ERK	Extracellular signal-regulated kinase
ESKD	End-stage kidney disease
GBM	Glomerular basement membrane
GFR	Glomerular filtration rate
GN	Glomerulonephritis
HD	Hemodialysis
HIF-1α	Hypoxia-induced factor-1α
HO	Heme-oxygenase
ICU	Intensive care units
LP	Lipid peroxide
L/P ratio	Lactate/pyruvate ratio
MAPK	Mitogen-activated protein kinase
MCP-1	Monocyte chemotactic protein-1
MDA	Malondialdehyde
MPO	Myeloperoxidase
NAC	*N*-acetylcysteine

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NF-κB	Nuclear factor-κB
OR	Odds ratio
OS	Oxidative stress
OSI	Oxidative stress index
PAN	Puromycin aminonucleoside
PICU	Pediatric intensive care units
ROS	Reactive oxygen species
rHuEPO	Recombinant human erythropoietin
TAC	Total antioxidant capacity
TGF-β	Transforming growth factor-β
TNF-α	Tumor necrosis factor-α
TPX	Total peroxide
VEGF	Vascular endothelial growth factor
ZnPP	Protoporphyrin IX zinc (II)

25.1 Introduction

Although it is accepted that reactive oxygen species (ROS) play a role in inflammation and tissue injury and there is clear evidence pointing to the potential utility of antioxidants in the treatment and prevention of kidney disease, the role of oxidative stress (OS) in the pathogenesis of acute and chronic kidney injury is still not completely understood and is the subject of much ongoing investigation.

In this chapter, several lines of experimental and clinical studies suggesting an important role of OS in the development of renal damage will be reviewed. The role of reactive nitrogen species in kidney disease, although an important subject, is not considered here due to space limitations.

25.2 Role of OS in Human Acute Kidney Injury

Acute kidney injury (AKI) is a clinical syndrome in which there is a rapid decrease in the glomerular filtration rate (GFR) and change in the homeostasis of the body. This is a significant problem in patients with critical illness and, indeed, approximately 5–6 % of all hospitalized adults suffer from varying degrees of AKI [1]. AKI is known to worsen mortality rates, increase duration of mechanical ventilation, and prolong hospital stays in critically ill adults and children [2, 3]. It is well known that AKI is associated with increased mortality and morbidity in critically ill children [4, 5]. Alkandari et al. recently reported that approximately 20 % of children admitted to pediatric intensive care units (PICU) developed AKI during admission and that AKI was associated with increased mortality (adjusted odds ratio (OR) = 3.7) [6]. AKI survivors are also at risk for progression to chronic kidney disease (CKD) [7]. The common causes of AKI are renal ischemia,

nephrotoxic medications, and sepsis followed by primary renal diseases and hemolytic uremic syndrome.

Evidence has accumulated incriminating ROS as the causative agent of renal damage in AKI, whatever the etiology may be [8, 9]. Cell injury occurs during reperfusion of ischemic tissues when molecular oxygen is introduced into the tissues [10]. ROS cause lipid peroxidation of cell and organelle membranes, leading to the disruption of structural integrity and capacity for cell transport and energy production, especially in proximal tubular cells within the kidney. These free radicals are short-lived and cannot be measured directly, but their activity can be measured by estimating the by-products and substances involved in defense against the oxidant injury. Biomarkers of ROS include malondialdehyde (MDA; a by-product of lipid peroxidation), protein carbonyl, nitrite, and trace metals such as copper, while defenses against ROS can be substances such as ascorbic acid, ceruloplasmin, and zinc [10]. Lipid peroxide (LP) and certain enzymes, such as superoxide dismutase and glutathione peroxidase, are increased in AKI patients, and LP has predictive ability in determining the outcome of these patients [11]. The role of ROS in the pathogenesis of AKI has been demonstrated mainly in experimental and animal studies [12, 13], while studies in humans, and especially in children, are scarce [14, 15].

Mishra et al. studied OS status in 40 patients with AKI aged 0–10 years in comparison with 20 age- and gender-matched healthy children [16]. In that study, plasma MDA, protein carbonyl, nitrite, copper, ascorbic acid, zinc, and ceruloplasmin levels were measured. They found that the plasma MDA, copper, ascorbic acid, and ceruloplasmin levels were significantly raised in AKI patients. Furthermore, the levels of plasma MDA, nitrite, copper, and ceruloplasmin were significantly higher in AKI nonsurvivors in comparison with survivors. The cutoff levels of plasma nitrite ($\geq$3.6 μmol/L) and ceruloplasmin ($\geq$127 mg/dL) were most accurate in predicting mortality in AKI patients and had maximum sensitivity (100 %) and specificity (60.7 %) among the parameters studied. Thus, they concluded that the increased levels of oxidants and antioxidants suggest a possible role of OS in AKI pathogenesis.

25.3 Mechanisms of AKI Development Involving OS

It appears that there are several mechanisms by which OS causes AKI. The following are postulated mechanisms in the development of AKI by OS.

25.3.1 Ischemia–Reperfusion

The healthy kidney generates physiologically moderate amount of ROS in the course of renal oxidative metabolism. The relatively low amount of ROS generated by the kidneys is tolerated without any apparent adverse effects. In contrast, in renal

ischemia, which is the most common cause of AKI in children, there is excessive production of ROS during reperfusion of the ischemic kidneys, and these ROS engender further renal injury by lipid peroxidation [13].

As it has long been known that ischemia–reperfusion of the myocardium also leads to a tremendous generation of ROS and cellular injury [17], the setting of elective surgeries such as cardiopulmonary bypass (CPB), in which the renal ischemia–reperfusion insult can be quantified prospectively, has been studied extensively. The incidence of AKI is high in patients undergoing cardiac surgery, reaching 50 % by some definitions [18]. Another study revealed that AKI after CPB is common and is associated with increases in morbidity, length of stay in the ICU and hospital, and mortality [19]. Based on these findings, ischemia–reperfusion is accepted as one of the important players in this type of renal injury, while it is clear that the pathogenesis of CPB-related AKI is multifactorial.

25.3.2 Macroscopic Glomerular Hematuria

Macroscopic hematuria is a common finding in various glomerular diseases, such as IgA nephropathy, Alport syndrome, and thin basement membrane disease [20], which do not necessarily show poor prognosis in terms of renal function. Although glomerular hematuria has been considered a clinical manifestation of glomerular diseases without real consequences on renal function and long-term prognosis, many of the studies performed have shown a relationship between macroscopic glomerular hematuria and AKI and have suggested that macroscopic hematuria-associated AKI is related to adverse long-term outcomes: up to 25 % of patients with macroscopic hematuria-associated AKI do not recover baseline renal function [21]. In cases with macroscopic hematuria-associated AKI, it has been speculated that pathophysiologic mechanisms account for the tubular injury found on renal biopsy specimens, i.e., mechanical obstruction by red blood cell casts was thought to play a role [22]. However, recent evidence points to cytotoxic effects of OS induced by hemoglobin, heme, or iron released from red blood cells, as shown in Fig. 25.1 [20]: the kidney can be damaged by large amount of heme resulting both from extrarenal heme-containing proteins (myoglobin in rhabdomyolysis and hemoglobin in hemolysis) [23, 24] and from renal heme-proteins, as occurs after ischemic or toxic insults [25, 26]. Intratubular, cell-free hemoglobin induces severe oxidative damage as a consequence of heme redox cycling between ferric and ferryl states, which generates radical species and promotes lipid peroxidation [27, 28]. Lipid peroxidation is responsible for the intense vasoconstriction and oxidative injuries observed in disorders associated with renal accumulation of hemoproteins [29]. Heme permeates plasma and organellar membranes, thereby entering the cells and facilitating cytotoxicity [30, 31]. Additional mechanisms of heme toxicity include impairment of the activity of certain glycolytic enzymes, such as glucose-6-phosphate dehydrogenase and glutathione reductase; oxidative DNA denaturation; and mitochondrial toxicity [32–35]. In addition to its direct cytotoxicity, heme can also indirectly promote

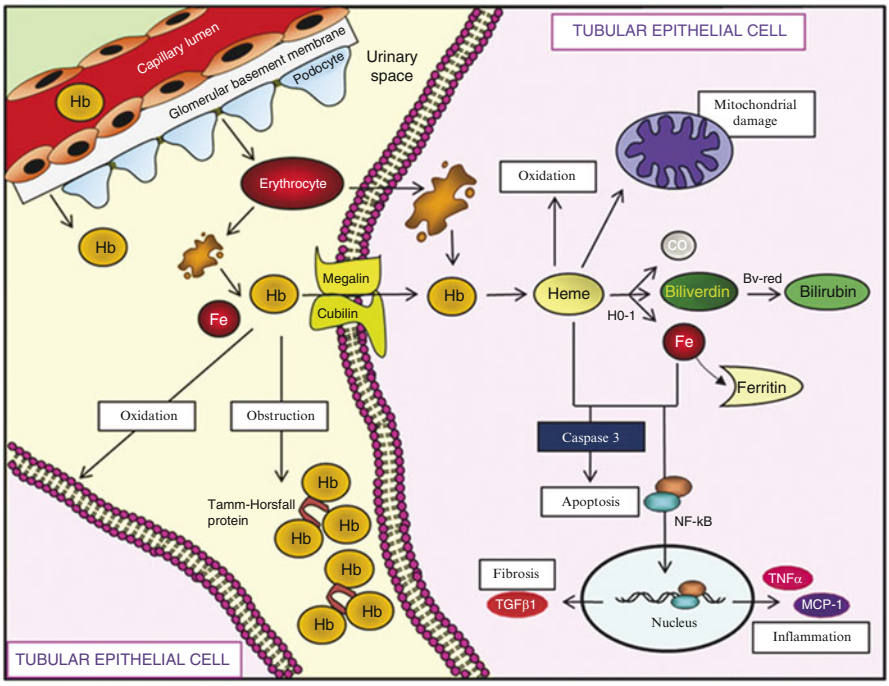

Fig. 25.1 Pathophysiologic pathways of hematuria-induced kidney damage. Hemoglobin (Hb) released by intratubular degradation of red blood cells or hemoglobin directly filtered by the glomerulus may be incorporated into proximal tubules through the megalin–cubilin receptor system or degraded in the tubular lumen, releasing heme-containing molecules and eventually free iron. Cell-free hemoglobin promotes lipid peroxidation and physical obstruction of the renal tubules by hemoglobin precipitation in association with Tamm–Horsfall protein under acidic conditions, which leads to intraluminal casts, increased intratubular pressure, and subsequent decreased GFR. Hemoglobin/heme/iron (Fe) accumulation within tubular cells generates reactive oxygen species, mitochondrial damage, caspase activation and apoptosis, upregulation of vascular adhesion molecules, and pro-inflammatory/profibrotic cytokines through activation of NF-κB transcription factor. Reproduced from [20] with permission from © 2012 by the American Society of Nephrology

chronic renal damage by inducing inflammation and fibrosis [36, 37]. Exposure to heme-proteins increases the renal expression of tumor necrosis factor (TNF)-β, monocyte chemotactic protein (MCP)-1, and transforming growth factor (TGF)-β via nuclear factor (NF)-κB transcription factor [23, 24]. The activation of these cytokine cascades serves as a "positive-feedback loop" that perpetuates renal damage beyond the initial injury phase [38] and contributes to a chronic inflammatory response, as occurs in recurrent hemolytic episodes [26]. These mechanisms of injury may be shared with hemoglobinuria or myoglobinuria-induced AKI.

Heme-oxygenase (HO) catalyzes the conversion of heme to biliverdin and is protective in animal models of heme toxicity. CD163, the recently identified scavenger receptor on the surface of the tissue macrophages, promotes the activation of anti-inflammatory pathways, opening the gates for novel therapeutic approaches [39].

25.3.3 Polymorphism of Genes in NADPH Oxidase p22phox and Catalase

Taking into consideration that genetic variation in the expression of pro- and antioxidant enzymes might account in part for the interindividual variability that is observed in the manifestation of acute organ injuries, including AKI [40, 41], Perianayagam et al. examined whether polymorphisms in the NADPH oxidase (p22phox +242C to T) and catalase (−262C to T) genes are associated with circulating biomarkers of OS in patients with AKI [42]. They measured plasma nitrotyrosine (a by-product of superoxide and nitric oxide generation) and whole-blood catalase activity. Recognizing that polymorphisms in these genes that code for NADPH oxidase p22phox and catalase have been shown to alter gene expression and enzyme activity, respectively, they tried to demonstrate the role of these variants in AKI patient outcomes. Through the prospective analysis of the DNA of 200 hospitalized subjects with AKI, they showed that those with a T allele at position +242 in the NADPH oxidase p22phox gene were at a twofold increased risk of requiring renal replacement therapy or hospital death. This association persisted even when controlling for age, race, gender, and severity of illness scores. In addition, it was demonstrated that patients with the NADPH oxidase p22phox TT genotype had significantly higher plasma nitrotyrosine levels as compared with those with the CC genotype. Similarly, patients with the catalase CT and TT genotypes had significantly lower whole-blood catalase activity as compared with those with the CC genotype. These results further lend support to the hypothesis that OS is involved in the pathogenesis of AKI.

25.3.4 Decreased Antioxidant Levels

Exercise-induced AKI in patients with idiopathic renal hypouricemia was first reported by Erley et al. in 1989 [43]. Since then, more than 50 cases have been reported, mostly from Japan [44]. It is speculated that patients with idiopathic renal hypouricemia have a 200-fold greater predisposition to exercise-induced AKI than those without renal hypouricemia [45]. Although the mechanism of exercise-induced AKI in renal hypouricemia is unclear, Murakami et al. emphasized the role of uric acid as an antioxidant [46]: renal handling of uric acid seems to be a protective mechanism against renal injury by free radicals. As both the uric acid pool and the total amount of uric acid mobilized into the proximal tubular cells are very small in patients with renal hypouricemia, renal perfusion is diminished during exercise, and reperfusion after exercise mimics ischemia–reperfusion renal injury.

Idiopathic renal hypouricemia is a rare disorder with the highest incidence of 0.15 % in Japan [47]. These patients have increased uric acid excretion due to an isolated defect in the renal tubular transport of uric acid arising from defects in a gene (*SLC22A12*) encoding the urate transporter 1 [48, 49]. As mentioned above, an

important complication of this disorder is exercise-induced AKI [44, 45]. The mechanism of AKI in this setting still remains unknown, though it is speculated that OS is involved [46, 50].

We first demonstrated the oxidative imbalance by a concomitant assessment of ROS production and antioxidant system capability in a 15-year-old girl with idiopathic renal hypouricemia caused by a mutation in the urate transporter 1 gene [51]. Her serum level of ROS increased with decreasing antioxidant potential capacity soon after the initiation of anaerobic stress due to treadmill exercise. Thereafter, the serum levels of ROS and antioxidant potential showed a parallel course, returning to the baseline values at 240 min after exercise. Therefore, it appears that some patients with idiopathic renal hypouricemia demonstrate oxidative imbalance soon after exercise with a predisposition to exercise-induced acute renal failure.

25.4 AKI Therapies Involving OS Pathway Modification

While much of the OS pathway has been well documented and its role in cell injury is becoming increasingly evident, further studies will be needed to improve the prophylactic and therapeutic interventions in the setting of AKI. In this section, the clinical and experimental trials of therapies involving alterations in the OS pathway are presented.

25.4.1 Ameliorating OS in Cardiac Surgeries

Haase et al. conducted a randomized multi-blind placebo-controlled trial of high-dose *N*-acetylcysteine (NAC) in high-risk patients undergoing CPB [52]. NAC can directly scavenge ROS and therefore is expected to reduce OS during CPB; it also regenerates the glutathione pool. In this trial, 61 subjects were randomized to administration of either 24 h of high-dose NAC (300 mg/kg body weight in 5 % glucose, 1.7 L) or placebo (5 % glucose, 1.7 L), with the primary end point being absolute change in serum creatinine within the first 5 postoperative days. The study failed to demonstrate a difference in the serum creatinine levels in this early postoperative period. Nouri-Majalan et al. performed a randomized trial investigating the role of supplemental antioxidant vitamin E and the inhibitor of xanthine oxidase allopurinol in reducing ischemia–reperfusion injury after coronary artery bypass graft surgery: patients were randomized to receive 100 units vitamin E four times per day and 100 mg allopurinol twice daily for 3–5 days prior to elective surgery, or to no treatment [53]. Their results showed that prophylactic treatment with vitamin E and allopurinol had no renoprotective effects in patients with preexisting renal failure undergoing surgery. Treatment with these agents, however, reduced the duration of the ICU stay.

In summary, although several studies have been conducted, no antioxidant agent has been shown to be of benefit for the prevention or treatment of AKI in patients undergoing cardiac surgeries [54].

25.4.2 Ameliorating OS in Drug-Induced AKI

Cisplatin is an important antineoplastic agent for the treatment of solid tumors, but its clinical use is limited because of its dose-dependent renal toxicity. Cisplatin nephrotoxicity involves OS, apoptosis, inflammation, and fibrogenesis. Ajith et al. conducted a comparative study of the effects of vitamin C and vitamin E on cisplatin-induced nephrotoxicity in mice [55]. Both vitamins have well-known antioxidant properties, and the experiment was designed to expose the animals to high and low doses of both vitamins C and E (250 and 500 mg/kg of each). The vitamins were administered 1, 24, and 48 h after cisplatin injection. Higher doses of both vitamins were effective in protecting against oxidative renal damage, as measured by increasing superoxide dismutase and reduced glutathione activity.

Several other agents have also been proposed as providing effective protection against cisplatin nephrotoxicity. In a rat model, the iron chelator deferoxamine has been shown to provide functional and histological protection [56]. Satoh et al. demonstrated that edaravone, a free radical scavenger, attenuated the cisplatin-induced mitochondrial membrane potential loss, decreased the concentrations of carbonylated proteins, and exhibited cytoprotective properties in murine proximal tubular cells [57].

Much as in the case of cisplatin nephrotoxicity, gentamicin-induced renal failure has been shown to respond to prophylactic and therapeutic measures in experimental animal studies. In vivo and in vitro, gentamicin enhances the generation of ROS by altering mitochondria respiration, leading to the generation of H_2O_2 [58]. Additionally, gentamicin enhances the generation of superoxide anion and hydroxyl radical by renal cortical mitochondria. Furthermore, gentamicin induces the release of iron from these renal cortical mitochondria; this causes lipid peroxidation in vitro, with iron serving as a potent catalyst for free radical formation. Kopple et al. investigated the role of L-carnitine, an antioxidant that prevents the accumulation of end products of lipid peroxidation, in the setting of a rat model of gentamicin-induced AKI and demonstrated that L-carnitine improved renal function and ameliorated the severity of renal pathologic findings in a dose-dependent manner [59].

Despite this success in experimental models and the likely tolerability of the treatments in humans, neither of these interventions has been translated into a clinical practice.

25.4.3 Ameliorating OS in Ischemia–Reperfusion-Induced AKI by Heme-Oxygenase Induction

While the strategy has not been tested in humans to date, there are compelling data from experimental animal models regarding the role of heme-oxygenase (HO) induction in preventing AKI by decreasing OS [60]. HO is an enzyme that is capable of converting heme into carbon monoxide, iron, and biliverdin, which is in turn reduced to bilirubin by biliverdin reductase (Fig. 25.1) [20]. This degradation of heme into carbon monoxide and bilirubin is considered to be protective against

OS-related tissue injury as both the reaction products are antioxidants. HO consists of two isoforms, HO-1 (inducible) and HO-2 (constitutive): HO-1 is induced in various cells by diverse stimuli that provoke OS, including heme, heat shock, pro-inflammatory cytokines, and toxins. Given these facts, HO-1 has been examined for its potential to reduce the impact of ischemia–reperfusion-induced AKI [61]. Wei et al. employed a mouse model of rhabdomyolysis, another important clinical cause of AKI due to the release of nephrotoxins (e.g., heme) from disrupted muscles, and demonstrated that the induction of HO-1 by granulocyte colony-stimulating factor led to a reduction in AKI, and these findings were reversed in the presence of an HO-1 inhibitor, protoporphyrin IX zinc (II) (ZnPP) [62].

25.5 Role of OS in CKD

CKD is a worldwide public health problem that affects approximately 10 % of the US adult population [63] and is associated with a high prevalence of cardiovascular disease [64] and high economic cost [65]. CKD, once established, tends to progress to end-stage kidney disease (ESKD). Given the notion that oxidants are one of the important players in inflammation, a strong association of ROS with CKD is easily conceivable. While evidence exists for the presence of increased OS in CKD in adults [66–69], such evidence is scarce in children.

Recently, Hamed et al. measured plasma hypoxia-induced factor-1α (HIF-1α), vascular endothelial growth factor (VEGF), total antioxidant capacity (TAC), total peroxide (TPX), pyruvate, and lactate in 40 pediatric patients with CKD on hemodialysis (HD) and 20 healthy children [70]. They demonstrated that TAC was significantly lower while TPX, the oxidative stress index (OSI) defined as the percent ratio of TPX to the TAC level, and VEGF were higher in patients both before and after a dialysis session than in the controls. Meanwhile, before dialysis, lactate/pyruvate (L/P) ratio was lower than after dialysis. In the data collected before the dialysis session, VEGF was positively correlated with pyruvate and HIF-1α, while OSI was positively correlated with TPX, but negatively correlated with TAC. After the dialysis session, HIF-1α was negatively correlated with TPX and OSI. From these findings, the authors postulated that CKD patients experience considerable tissue hypoxia in response to OS. HD ameliorated hypoxia but lowered antioxidant levels, as evidenced by the finding that the levels of HIF-1α and TAC were lower before than after dialysis.

25.6 Mechanisms of OS-Related Renal Damage in the Progression of CKD

A sufficient body of in vitro and in vivo information exists to postulate that oxidants are important mediators in progressive kidney disease. Although there is little information on the mechanisms of OS-related renal damage in CKD in humans, we discuss a possible mechanism in the following section.

25.6.1 Renal Damage Due to ROS Produced by Underlying Conditions for CKD

One reason for OS in patients with CKD is the underlying disease itself [70]. That is, renal toxicity and immunological disorders causing kidney diseases result in an elevated formation of ROS which further deteriorate the renal function. In addition, treatment procedures for patients with CKD have also been shown to induce OS. During HD, for example, incomplete correction of the uremic toxicity together with the untoward effects of dialysis and malnutrition and the progressive worsening of the clinical condition can lead to OS. The bioincompatibility of dialysis membranes represents an important source of ROS. Losses of antioxidants via dialysis are the factors that may be responsible for the imbalance between prooxidative and antioxidative mechanisms in HD patients [71]. Furthermore, TAC in patients with renal failure was shown to be greatly diminished due to antioxidant exhaustion and inhibition [72]. The activation of neutrophils and the complement pathway during an HD session as the result of interactions of the blood with the dialysis membrane and endotoxin-contaminated dialysate, iron overload, the presence of advanced glycation end products, high homocysteine levels, intradialytic cytokine activation, or other causes could also play a role [73].

25.6.2 Excessive Protein Trafficking Through the Glomerulus

It is well known that the severity of renal tubulointerstitial injury is a major determinant of the degree and rate of progression of CKD. There has been increasing interest in the possible link between excessive protein trafficking through the glomerulus and progressive tubulointerstitial inflammation that leads to CKD, as shown in Fig. 25.2 [74]. A candidate key molecule for chemokines induced by enhanced protein uptake by renal tubular cells is NF-κB: 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 interleukin-8, which are important in the inflammatory response [75, 76]. Morigi et al. have shown that human proximal tubular cells incubated with human albumin and IgG lead to a significant and rapid increase in H_2O_2 and activation of NF-κB. Furthermore, inhibitors of protein kinase C significantly prevented H_2O_2 production and consequent NF-κB activation [75].

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 the diseased kidney [77]. It has been reported that ROS mediate TGF-β1-induced EMT in renal tubular epithelial cells directly through the activation of mitogen-activated protein kinase (MAPK) and indirectly through extracellular signal-regulated kinase

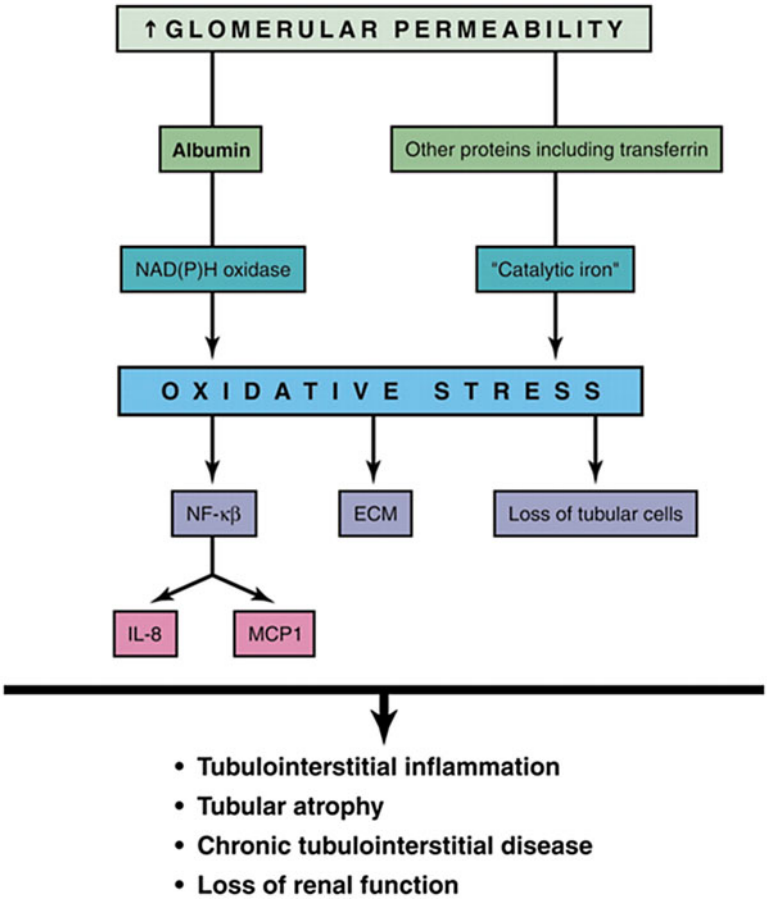

Fig. 25.2 Relation of proteinuria to oxidant stress and tubulointerstitial injury. Reproduced from [74] with permission from © 2007 by the American Society of Nephrology

(ERK)-directed Smad 2 phosphorylation, and it has been suggested that antioxidants and MAPK inhibitors may prevent EMT through these pathways and ameliorate subsequent tubulointerstitial fibrosis [78].

Another pathway to OS is speculated to be related to iron (Fig. 25.2). The data support the role of iron in models of progressive renal disease consisting 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 in this model [79]. Similarly, it has been shown that there is a substantial iron accumulation associated with increased cortical MDA in proximal tubular cells in the remnant kidney, suggesting

ROS generation. The sources of increased iron in the kidney have not been well delineated, although one group postulated that urinary transferrin provides a potential source of iron [80, 81].

25.6.3 Evidence of the Involvement of OS in Renal Injury in Animal Models

Diverse pathogenetic roles of oxidants in progressive kidney disease have been demonstrated by experimental studies [74]. Experimental glomerulonephritis (GN) can be classified into two categories, i.e., leukocyte-dependent GN and leukocyte-independent GN. The former is further categorized into two well-studied models, the anti-glomerular basement membrane (anti-GBM) antibody model and the anti-Thy 1.1 model mimicking mesangial proliferative GN, while the latter is further categorized into a puromycin aminonucleoside (PAN)-induced nephrosis model, a model of minimal-change disease, and passive Heymann nephritis induced by anti-tubular brush-border antiserum (anti-Fx1A), a model of membranous nephropathy. Although leukocytes have not been considered to be important in animal models of membranous nephropathy, there is evidence for the potential participation of a myeloperoxidase (MPO)–H_2O_2–halide system in membranous nephropathy [82, 83]. Thus, it appears that leukocytes or resident glomerular cells serve as sources for oxidants in this model. The reported roles of ROS in these experimental GN are summarized in Tables 25.1 and 25.2 [74].

25.7 Therapeutic Interventions of CKD by Modulating OS

Recently, a meta-analysis on the efficacy of antioxidant therapy in patients with CKD was reported [84]. This study included ten randomized controlled trials consisting of 1979 participants, and assessed the beneficial effects of antioxidant therapy on cardiovascular events and development of ESKD. The results showed that antioxidant therapy with vitamin E, multiple antioxidant therapy, or treatment with coenzyme Q, acetylcysteine, bardoxolone methyl, or human recombinant superoxide dismutase showed no clear overall effect on cardiovascular events such as mortality. However, some of the treatments were of significant benefit for reducing the development of ESKD. The authors concluded that antioxidant therapy in predialysis CKD patients may prevent progression to ESKD, although it does not reduce the risk of cardiovascular events. An appropriately powered study with a longer follow-up is awaited.

A novel agent that gives us hope is erythropoietin (EPO). EPO is the principal hematopoietic hormone produced by the kidney and the liver, and has been shown to regulate mammalian erythropoiesis and to exhibit diverse cellular effects in

Table 25.1 Experimental evidence for the role of oxidants in leukocyte-dependent glomerulonephritis

Leukocytes as a source of oxidants for glomerular injury
• A wide variety of soluble and particulate stimuli, including immune complexes, complement components [90], and ANCA[a] [91]
• Anti-GBM[b] 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 glomerulonephritis [92]
• Enhanced superoxide and hydroxyl radical are generated by macrophages that are isolated from glomeruli of rabbits with anti-GBM antibody disease [93]
• Enhanced superoxide generation by macrophages that are isolated from nephritic glomeruli (anti-thymocyte serum) [94]
Effects of oxidants that are relevant to occurrence of proteinuria in glomerular injury
• Oxidants participate in GBM[b] degradation [95]
• Infusion of myeloperoxidase-H_2O_2 induces proteinuria [96]
Evidence for the role of oxidants in animal models
• Catalase markedly reduces proteinuria, whereas superoxide dismutase has no protective effect in anti-GBM[b] antibody disease [97]
• A hydroxyl radical scavenger and an iron chelator significantly attenuate anti-GBM antibody-induced proteinuria in anti-GBM[b] antibody disease [98]
• Alpha-lipoic acid is protective in an anti-Thy 1.1 model [99]
• Liver-type fatty acid-binding protein protects mice with anti-GBM glomerulonephritis from progression of both tubulointerstitial and glomerular injury by acting as an antioxidant [100]

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[a]ANCA, antineutrophil cytoplasmic antibody

[b]GBM, glomerular basement membrane

non-hematopoietic tissues [85]. EPO significantly protects multiple organs in both acute and chronic diseases [85, 86]. Patel et al. reported that EPO was able to significantly attenuate the renal dysfunction and injury associated with ischemia/reperfusion in mice [87]: the increase in renal MPO activity (as a surrogate for polymorphonuclear leukocyte infiltration) and tissue MDA levels (as a surrogate for tissue lipid peroxidation) were also significantly reduced in EPO-treated mice. Bahlmann et al. demonstrated that chronic treatment with the long-acting recombinant human EPO (rHuEPO) analogue darbepoetin alpha conferred renal vascular and tissue protection and preserved renal function in the established 5/6 nephrectomy remnant kidney model in the rat, a model that features progressive injury leading to glomerular sclerosis and ischemia-induced tubulointerstitial damage [88]. Treatment with rHuEPO not only reduced renal dysfunction but also significantly improved the survival of uremic rats. In this experimental setting, the authors observed persistent activation of the Akt pathway in endothelial and epithelial glomerular cells, and reduced apoptotic cell death in renal tissue. Importantly, they used a hematologically noneffective dose of darbepoetin which did not affect hematocrit levels in treated animals. This

Table 25.2 Experimental evidence for the role of oxidants in leukocyte-independent glomerulonephritis

In a PAN[a] model of minimal-change disease
• Cultured glomerular epithelial cells exhibit an enhanced generation of H_2O_2 [101]
• Administration of scavengers of oxidants and antioxidants results in reduction in proteinuria [102–105]
• A novel free radical scavenger, edaravone, delays and ameliorates the urinary protein excretion in rats [106, 107]
• Glomerular catalytic iron is increased [108], and cytochrome P450 is an important source of the catalytic iron [109–111]
• Feeding with a selenium-deficient diet results in a marked diminution of glutathione peroxidase accompanied by an increase in proteinuria [112]
• Inhibition of superoxide dismutase by diethyldithiocarbamate results in increase in PAN[a]-induced proteinuria [113]
• Induction of antioxidant enzymes by ischemia–reperfusion injury protects against H_2O_2-induced proteinuria [114]
• Induction of antioxidant enzymes by glucocorticoids protects against PAN[a]-induced proteinuria [115]
• Apocynin, an inhibitor of NADPH oxidase, decreases superoxide production in podocytes, and inhibits endocytosis and urinary albumin excretion [116]
Evidence for the role of oxidants in passive Heymann nephritis
• There is an increased generation of H_2O_2 in passive Heymann nephritis [117]
• In a passive Heymann nephritis, a model of membranous nephropathy, hydroxyl radical scavengers and an iron chelator and probucol significantly reduce proteinuria [118, 119]
• Feeding with an iron-deficient diet results in a reduction in proteinuria [120]
• Feeding with a selenium-deficient diet results in marked diminution of glutathione peroxidase in anti-Fx1A-induced proteinuria [112]

[a]PAN, puromycin aminonucleoside (Reproduced with slight modification from [74] with permission from © 2007 by the American Society of Nephrology)

could be of considerable clinical relevance, since "low-dose" rHuEPO treatment may be a safe strategy to avoid potential adverse effects of "high-dose" rHuEPO with a risk of an increase in hematocrit with concomitant hyper-viscosity and activation of thrombocytes [88].

Fujiwara et al. recently observed that 15 CKD patients with anemia treated with rHuEPO (12,000 U administered subcutaneously once every 2 weeks) showed increased serum hemoglobin levels and decreased urinary levels of protein, liver-type fatty acid-binding protein (a biomarker of renal injury), and 8-hydroxydeoxyguanosine (a biomarker of OS) after 6 months, while serum creatinine and estimated GFR showed little difference throughout the experimental period [89]. The authors concluded that rHuEPO may ameliorate renal injury, OS, and progression of atherosclerosis in addition to improving anemia in CKD patients. It is therefore conceivable that therapy with rHuEPO may join the arsenal of approaches directed against progressive CKD.

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