Cellular Oxidative Processes in Relation to Renal Disease

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Key Words

Advanced glycation end-products • Cell signal transduction • Glomerulonephritis • Glutathione • Hypertension • Nitrogen species • Nuclear factor κ B • Oxidative stress • Proteinuria • Reactive oxygen species

Abstract

This article summarizes the biochemical processes that produce reactive oxygen species (ROS) and other mediators that account for 'oxidative stress'. Formation of ROS in signal transduction cascades is illustrated from studies of kidney cell systems. The pathophysiological implications for the nephrologist are then reviewed.

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Introduction

Reactive oxygen species (ROS), which are highly reactive by virtue of one or more unpaired electrons in their outer orbits, are generated endogenously in metabolic pathways. 'Oxidative stress' reflects an imbalance between the formation of ROS and the antioxidant defences. ROS arise from (i) the mitochondrial electron transport chain as part of oxidative phosphorylation; (ii) the metabolism of arachidonate by cyclooxygenase or lipoxygenase enzymes to prostaglandins or leukotrienes; (iii) cytochrome P₄₅₀ enzymes; (iv) oxidase enzymes like NADPH oxidases, or (v) the nitric oxide synthetases. Small physiological amounts of ROS are required in sig-

nalling pathways and so cells are activated. ROS formation is so much greater when cells are receiving stress signals. Overproduction of ROS and nitrogen species can cause significant cellular damage. Accordingly cells possess various antioxidant systems. Thus superoxide can be dismutated by superoxide dismutase (SOD) to yield hydrogen peroxide, but that H₂O₂ may be converted to the hydroxyl radical ($\cdot$ OH) which will interact with proteins, lipids or nucleic acids. When there is myeloperoxidase of neutrophils, that H₂O₂ forms additional oxidants.

Cell signaling involves post-translational modification of proteins at redox centres which become reduced by gaining electrons or oxidised by losing electrons. A common redox reaction is the breaking or formation of a protein disulphide bridge [1]. If there is oxidative stress, there is permanent formation of disulphide bridges (-S-S-) and so changes in protein structure. The situation is detected as loss of reduced glutathione GSH by its conversion to GSSG, since GSH and thioredoxin maintain signal molecules in the reduced state. The amino acid that is implicated most often is cysteine, and it is modified by ROS and by reactive nitrogen species (RNS). A single cysteine can present in four different forms, as reduced RSH, oxidised RSOH, S-nitrosylated RSNO or the glutathionylated RSSG [1]. The sequence Cys-X-X-Gly-X-X-Arg-Ser/Thr is a signature motif in certain protein tyrosine phosphatases. Since signal transmission takes place through phosphorylation of proteins, the dephosphorylations by means of phosphatases exert control, and their redox modulation makes sense [2]. Whenever there is inactivation of protein tyrosine phosphatases by ROS, there

Table 1. Agents which induce ROS production in target cells

Angiotensin II	bradykinin
Arachidonic acid	thrombin, complement C5a
Insulin growth factors	EGF, FGF, PDGF, TGFβ
Cytokines	IL-1β, TNFα, IFNγ
Calcium ionophores	phorbol esters

will be prolonged activation of specific stress-responsive or mitogenic signaling pathways.

During protein folding in the endoplasmic reticulum of cells, an oxidase Ero1p generates an intermediate disulphide to oxidise target thiol proteins by thiol/disulphide exchange. Likewise cysteine generates the disulphide cystine extracellularly. In fact, the cystine/cysteine redox state of plasma is more oxidised relative to GSSG/GSH, and varies independently of that [3].

Cysteine and methionine are especially prone to oxidative attack. There can also be oxidation of Lys, Arg, Pro, Thr or other residues that lead to formation of protein carbonyl (–C=O) derivatives, i.e. aldehydes and ketones. Since such carbonyls are more difficult to induce, albeit they arise from metabolism of carbohydrates, lipids and amino acids, they indicate a more severe oxidative stress [4]. Protein carbonyls accumulate during age-related diseases and *advanced oxidative protein products* (AOPP) are elevated in uremic patients. Protein carbonyls are non-specific, but conversion of tyrosine residues to 3-chlorotyrosine, 3-nitrotyrosine and dityrosine can be considered better markers of oxidative stress. Dityrosine-crosslinked protein products are the AOPPs. Tyrosine can be modified by HOC1 generated by myeloperoxidase or by ONOO[–] to form nitrotyrosines. The products are irreversible [5] and they are demonstrable in tissues.

Carbonyl groups can also arise in proteins by addition reactions of α,β-unsaturated aldehydes such as 4-hydroxy-2-nonenal, malonyldialdehyde and acrolein (2-propenal) [6]. Indeed one is now cognisant of *lipid peroxidation products* such as thiobarbituric-acid-reactive substances (TBARs), like malonyldialdehyde, acrolein, 4-hydroxynonenal [7], all appropriately listed in [8], and the 18 monohydroxyfatty acids, isolevoglans and F2-isoprostanes [9] and oxidised LDL. The *carbohydrate oxidation products* are referred to as advanced glycosylation end-products (AGEPs), which are diverse fluorescent and non-fluorescent compounds formed by non-enzymatic reaction of reducing sugars or other carbonyl compounds with free amino groups of proteins [8]. Finally, there are

products of *nucleic acid oxidation* like 8-hydroxy-2-deoxyguanosine.

One could be amazed at the variety of agents which can induce ROS in their particular target cells as shown in table 1. Accordingly many physiological reactions are naturally controlled by release of small amounts of ROS [10, 11].

Consider a familiar example: when angiotensin (Ang) II acts on vascular smooth muscle cells (VSMs), it increases oxidative stress via activation of the NADH/NADPH oxidases at the VSM membrane [12]. So there have been trials to assess whether antioxidants like vitamin E can control vascular disease, in particular coronary artery disease [13] in chronic renal failure patients [8]. SOD defends against ROS. Yet it actually converts superoxide anions O₂[–] that cannot penetrate cell membranes to H₂O₂ that does. Hence, SOD that is bound to heparin sulphate glycosaminoglycans on endothelial cells might transduce extracellular oxidative stress to the interior of cells. This will be relevant to vascular pathology [8, 14].

The counteracting redox molecules in the cytosol of cells are pyridine nucleotides 1–2 mM, lactate: pyruvate 1–2 mM, cysteine thiols 25 mM and glutathione 1–10 mM. The glutathione GSH-GSSG system is paramount for protection against oxidative threats. It is reinforced by plasma and intracellular ascorbate [15]. There is back-up by the antioxidant enzymes SOD, catalase and glutathione peroxidase [16, 17]. The heat shock proteins often act as SODs [18]. So does the tissue antioxidant metallothionein, which also protects against nitric oxide. Related to it is the intracellular redox regulating agent thioredoxin, a free radical scavenger that removes H₂O₂, and which helps control over nuclear factor (NF)-κB [19].

The cytokine macrophage migration inhibitory factor is expressed by immune and non-immune cells, and it too acts as a regulator of cellular redox stress. In fact macrophage migration inhibitory factor protects cells from pro-oxidative-stress-induced apoptosis [20].

Reactive Nitrogen Species

Conversion of *L*-arginine to NO requires two monooxygenase reactions. So NO synthases are linked to oxidative processes. It is now well understood that there is a link between formation of ROS at the vascular endothelium, and endothelial dysfunction and vascular damage [13]. The endothelium is both a source and a target of ROS. Local superoxide will inactivate endothelial nitric

Fig. 1. ROS-activated signal pathways. Asterisks indicate redox-sensitive steps that are sensitive to ROS. PKC = Protein kinase C.

oxide synthase. Also superoxide reacts with NO to form peroxynitrite $ONOO^-$ which is damaging to tissues. Peroxynitrite mediates Ca^{2+} -dependent mitochondrial dysfunction via activation of calpain. Carbon dioxide reacts with $ONOO^-$ to form NO_2 and the carbonate radical (CO_3^-). These two radicals nitrate tyrosine residues in proteins, albeit that reaction is blocked extracellularly by urate. Within cells NO-dependent protein nitration mediates catalytic activity, cell signalling and cytoskeletal organization [21].

Oxidations by ROS and Signal Transduction

Free radicals like $O_2^{\cdot}$ or nitric oxide $NO^{\cdot}$ often initiate or help drive signalling cascades [22], as in mesangial cells. Details appear in figure 1. Firstly, when agents like those shown in table 1, e.g. PDGF or EGF, bind to their receptors, there is transient formation of H_2O_2 which helps signal transduction. The ROS then affect the G-proteins Ras, Rho and Rac-1. Secondly, ROS or nitric oxide will act as second messengers at various stages of cell signal transduction. Oxidative modification of proteins affects redox-sensitive amino acids, like cysteine or histidine [1] causing conformational changes in protein tyrosine kinases or their balancing phosphatases [23] so altering phosphorylation/dephosphorylation reactions. H_2O_2 that is produced by transactivation of surface growth factor receptors activates Src kinases (or Akt) and thereby ERK1/2 of the mitogen-activated protein kinases (MAPK) [24]. The stress-activated protein kinases desig-

nated JNK and p38 MAPK respond to ROS, and so they can exert influence on genes. Thirdly, ROS regulate gene expression [25], e.g. when H_2O_2 activates NF- κ B DNA binding protein, or when AP-1 (c-fos, c-jun) is activated. As the scheme for transforming growth factor- β (TGF β) in figure 1 indicates, oxidative processes driven by AngII also play a part in renal fibrosis [26]. ROS act as intermediaries in TGF β -mediated deposition of extracellular matrix proteins [27]. In part ROS activate calcineurin to help in induction of fibrosis.

With respect to renal pathophysiology, at the present time the literature deals with a variety of cell types and one has to present the general concept by using diverse illustrations rather than by hoping to find all the examples from the study of mesangial cells or podocytes, etc. When a growth factor or cytokine, or AGEs, act on cell surface receptors, they dimerize and become phosphorylated in order to trigger signalling cascades, starting with activation of phosphotyrosine kinases like the Src-kinases. As PDGF acts on mesangial cells, there is transient generation of H_2O_2 which requires the support of phosphatidylinositol 3-kinase [28], and Src-kinases are activated [24]. Whenever interleukin (IL)-1 induces MAPK in mesangial cells [29], the process requires a transient burst of compartmentalised oxidation, and likewise when tumor necrosis factor- α ($TNF\alpha$) acts on mesangial cells [30]. Soon it was shown that the electron transport chain of mitochondria must be the source of ROS for the action of $TNF\alpha$. It was demonstrated too that $TNF\alpha$ -induced generation of ROS presages expression by mesangial cells of monocyte chemotactic protein, MCP-1, and of macro-

phage colony-stimulating factor [31]. Inevitably immune-mediated injury of mesangial cells is accompanied by ROS production [32] and leads to generation of eicosanoids, i.e. ROS are implicated in Cox-2 expression [33]. Soberman and Christmas [34] have pointed out that generation of lipid hydroperoxides within or near the cell nucleus as a result of the action of lipoxygenases must help regulation of transcription. Indeed 5-LOX enzyme contributes to the generation of ROS that activate NF- κ B.

It is now recognised that oxygen radicals play a vital role in NF- κ B activation [35] that leads to activation of genes for cytokines and chemokines, adhesion molecules, acute phase proteins, etc. [36]. Actually NF- κ B has separate oxidant-initiated and redox-regulated steps [37]. Hence N-acetylcysteine is found to reduce NF- κ B activation and to block display of VCAM-1 on endothelial cells [38]. Leading up to NF- κ B activation there is the PI 3-kinase: protein kinase B (Akt) pathway that is redox sensitive, or the p21Ras-Raf.1-MAPK cascade that has redox-sensitive steps [39] (fig. 1). As mentioned, the parallel JNK [40] and p38 MAPK pathways are sensitive to oxygen radicals. They are controlled by glutathione.

Control by Glutathione and Antioxidants

Intracellular GSH is essential for defence of cells against oxidants and toxic stress [15, 18]. Control over synthesis is important in most cells. One might expect glutamine, cysteine and glycine to be taken up by the brush border of renal tubular cells, but there is a limited availability of cysteine by uptake, and proximal tubular cells are hampered in their ability to synthesize GSH. Instead they rely on uptake of glutathione from extracellular fluid via their basolateral membranes [41]. So they have limited defence against chemical injury or oxidant stress. There is adequate GSH for the various redox controls that are part of normal metabolism. Importantly, GSH regulates the sphingomyelinase that produces ceramide that can mediate TNF α -induced cell death [42] as in the renal tubular cells [43]. In adriamycin nephrosis, which produces minimal change glomerular damage akin to that caused by purine aminonucleoside, there is generation of superoxide anions and hydroxyl radicals. Dimethylthiourea which sustains GSH levels ameliorates the situation. Likewise in Heymann nephritis in rats complement activation induces NADPH oxidoreductases in podocytes so causing production of oxygen radicals at the glomerular basement membrane [44].

Oxidation and Subcellular Controls

Cells are compartmentalised. Particular parts of the cellular apparatus may be more susceptible to oxidative damage. Cell redox status affects ubiquitination [45]. The study of neurodegenerative diseases reveals that oxidative stress causes dysfunction of proteasomes so that damaged proteins may not be removed by ubiquitination and proteolysis, and aggregated proteins accumulate in the cytosol. It is quite possible that such a process could be identified in the proximal tubules of the kidneys!

We still have much to learn about isoprostanes [6] and the cyclopentenone prostaglandins. At low concentration, the latter generate ROS and they are proinflammatory [46]. Plasma and urine levels of F2-isoprostanes are elevated during diabetes and in the course of glomerulonephritides. F2-isoprostanes increase the production of endothelin (ET)-1 by endothelial cells. In streptozotocin-induced diabetic rat kidneys increased isoprostane synthesis relates to ET-1 mRNA expression by endothelial cells and mesangial cells [47]. Hence one notes that antioxidants can be used to inhibit ET-1-induced proliferation of VSMs since they will inhibit MAPK pathways and the activator protein AP-1 [48].

Nephrological interest is focussed upon apoptosis, which is often consequent upon oxidative stress. Depletion of cell GSH causes the release of cytochrome-C from mitochondria even in the absence of a commitment to apoptosis [49]. Loss of redox balance as a prelude to apoptosis is shown in studies of oxidant formation by the non-fluorescent probe dihydrorhodamine-123 [50]. Initially this is independent of ROS formation. Yet when loss of cytochrome-C from mitochondria leads to disruption of electron transport, there is ROS formation. Loss of the mitochondrial transmembrane potential is the point of no return in the determination of apoptosis, for then oxidation of Apaf-1 leads to activation of caspases. The oxidation of phosphatidylserine leads to its externalisation on cell membranes [51] and such cells are recognised by macrophages for clearance. Cell death is a prominent feature of systemic lupus erythematosus, probably on account of anomalies of cellular NF- κ B controls. The T lymphocytes of patients with systemic lupus erythematosus show persistent mitochondrial hyperpolarisation and increased formation of ROS [52]. Generally in T cells Rap.1 signaling is required for suppression of Ras-generated ROS [53].

Pathophysiological Implications

Hypertension and Vascular Disease

This review commenced with emphasis that NAD(P)H oxidases are major sources of superoxide anions in VSMs. Either AngII or aldosterone or the mechanical pressure within arteries of the hypertensive patient activates these NADPH oxidases [12], which have Nox1 and Nox4 components. The vasoconstriction caused by infusion of AngII is attenuated by ascorbic acid or by tempol SOD mimetic [54]. Otherwise production of oxygen radicals causes endothelial cell dysfunction and there is oxygen-radical-induced degradation of NO [55], specifically in the renal medulla. Generally there is reduced endothelium-dependent vascular relaxation [13]. In spontaneously hypertensive rats the defective endothelium-dependent vasorelaxation to acetylcholine can also be ameliorated by a SOD mimetic [56]. Aortic rings of those animals show staining for nitrotyrosine that is reduced by SOD.

Circulating endothelial cells can now be used as an indicator of oxidative stress. Oxidant stress is implicated too in the expression of tissue factor and thus in the initiation of coagulation [57]. AngII infusion leads to adherence of leukocytes within arterioles. When AngII acts on neutrophils, there is an increase of both intra- and extracellular ROS production from translocation of the phagocyte NADPH oxidase to the cell membrane, and there is rapid phosphorylation and activation of ERK1/2, JNK1/2 and p38 MAPK, and activation of cell NF- κ B [58]. Both adhering leukocytes and VSMs are sources of ROS that lead to contraction of VSMs [59] and subsequent vascular growth [60]. The action of AngII can cause apoptosis in renal proximal tubule cells via a process that involves ROS and release of TGF β [61].

Giner et al. [62] have shown evidence for oxidative stress in subjects with essential hypertension, especially when there is microalbuminuria. The GSSG/GSH content of peripheral blood mononuclear cells of patients was elevated, as was the plasma malonyldialdehyde and the urinary excretion of 8-oxo, 2-deoxyguanosine.

Ischemia-Reperfusion in Acute Renal Failure and Sepsis

Ischemia-reperfusion leads to microvascular permeability and protein leakage in the kidney in acute renal failure, and accumulation of monocyte-macrophages [63] and neutrophils. The return of oxygenated blood leads to production of oxygen free radicals [64]. The consequent endothelial cell injury and subsequent inflammation provoked by leukocytes correlates with processing of endo-

thelial monocyte activating polypeptide II [65]. 5-Lipoxygenase metabolites enhance the ischaemia-reperfusion process and promote adhesion molecules within vessels. Formation of ceramide contributes to death of renal tubular epithelial cells [44] and oxidation enhances loss of tubular cell membrane phospholipids [66].

Oxidative stress in critically ill patients with systemic inflammatory response syndrome is evident from raised plasma malonyldialdehyde and 4-hydroxynonenal, the lowered thiol-bearing glycoproteins, the elevation of nitrates and nitrate, and the release of myeloperoxidase and elastase [67]. Multi-organ failure is a common sequel which implies ischaemia-reperfusion injury to vital organs along with microcapillary thromboses.

Oxidative Processes in Glomerulonephritides

Following the surge of publications on the biological importance of oxygen radicals came the reviews by Shah [68] on their role in experimental glomerulonephritis. In anti-Thy.1 rat glomerulonephritis, Budisavljevic et al. [69] showed how ROS enhance cell proliferation and phenotypic transformation via MAPK, and matrix accumulation and fibrosis via TGF β , and how all this can be ameliorated by the antioxidant α -lipoic acid. Recent studies show that oxygen radicals induce the maturation of dendritic cells [70] and that SOD plays a role in immune regulation [71]. The role of cell Fc receptors for clearance of immune complexes has been stressed of late. In nephrotoxic nephritis the infiltrating polymorphonuclear leukocytes induce glomerular TNF α expression via Fc-receptor-mediated H₂O₂ production [72]. In IgA nephropathy the plasma AOPP as a measure of oxidative stress has been shown to reflect prognosis [72b].

Determinants of antigens, the epitopes, are formed when changes in local charges alter protein conformation. Deacetylation and dephosphorylation of proteins, or citrullination, or methylation can all create neoepitopes. So can oxidation, as when ROS acting on glutamic acid decarboxylase induces immunogenic epitopes that lead to diabetes [73], or when hydroxyl radicals interact with chromatin to produce altered nucleosomes [74]. In systemic lupus erythematosus 8-hydroxyguanosine is detectable within immune complexes. Antibodies have an intrinsic ability to catalyse oxidation of water to ROS, which helps when they have to destroy pathogens [75], but may generate adverse immunological effects. In anti-glomerular basement membrane nephritis in man is the target antigen native α 3(IV)NC1 or does hydrocarbon exposure or cocaine abuse engender chemical alteration? Does the action of antibody also spread the response?

In most glomerulonephritides there is an interstitial inflammatory infiltrate that generates oxidants and AngII and other vasoconstrictors that could account for the sodium retention of nephrotic syndromes [76]. Oxidants could lead to further sodium sensitivity as a result of local inactivation of NO [77]. Indeed Rodriguez-Iturbe et al. [78] have shown evidence that the oxidising effects of interstitial immune cells and release of AngII can create salt-sensitive hypertension. There should be means like heme oxygenase action that will curb the effects of oxidants, certainly in the context of haematuric glomerulonephritides [79]. We know now that the anti-inflammatory effect of IL-10 is mediated in part by HO-1 [80].

Vasculitides

Anti-neutrophil cytoplasmic autoantibodies activate monocytes and neutrophils with the release of ROS [81] and release of myeloperoxidase that uses H₂O₂ to create nitrating oxidants [82]. The oxidative process helps exposure of phosphatidylserine on antibody-damaged neutrophils, so that they are phagocytosed [83]. Yet actually chloramine dampens this process [84] and could explain the impaired neutrophil clearance [85], which will mean that antigens could persist to induce autoantibody formation. However, excessive ROS production by neutrophils can also lead to hyporesponsive T lymphocytes [86].

Proteinuria

Exposure of proximal tubules to albumin in urine causes the release of chemokines Rantes and MCP-1. They are mainly secreted toward the basolateral compartment and thus will be positioned to attract macrophages and to initiate tubulointerstitial fibrosis. Albumin uptake by tubular cells activates their protein kinase C, and there is formation of ROS which activates NF- κ B so releasing those chemokines [87]. By the use of diphenyliodonium it was demonstrated that a membrane NADPH oxidase is the source of the ROS [88]. It is suggested [89] that urine albumin is oxidatively modified, so producing reactive carbonyl groups, and that it carries oxidised lipids. Fatty acids bound to albumin will undergo auto-oxidation to yield reactive carbonyl compounds. Thus it could activate inflammatory cascades. Systemic oxidant stress would explain why in children with nephrotic syndrome the antioxidant status of white cells is lowered and their synthesis of IL-2 is increased by 30% [90].

AGEPs and Diabetic Nephropathy

Hyperglycaemia leads to the formation of superoxide anions and nitric oxide [91]. Hyperglycaemia accelerates

the synthesis and tissue deposition of AGEPs. These AGEPs create oxidative stress and lipid peroxidation and so they play a significant role in the pathogenesis of vascular and renal complications associated with diabetes [91]. There is also AGE formation even early in uremia on account of oxidative stress [92]. 3-Deoxyglucosone accumulates in uremic serum and reacts with protein amino groups to form AGEs. When AGEPs interact with cell surface receptors called RAGEs, they induce oxidant stress in various cell types [93], and that leads to NF- κ B activation and RAS-dependent pathway activation. RAGEs on lymphocytes and macrophages explain proinflammatory effects like the release of cytokine IL-2, interferon- γ and IL-1 β , respectively [94]. Interaction of AGEPs with mesangial cells leads to oxidative upregulation of a number of stress-related genes like NF- κ B and protein kinase C- β 1 [95]. There is a case for the use of antioxidants in the prevention of diabetic nephropathy. High glucose can cause mesangial cell apoptosis via oxidative processes [96]. AGEPs via stimulation of protein kinase C and TGF β stimulate collagen synthesis in mesangial cells [97]. AGEPs stimulate tubular epithelial: myofibroblast transitions via RAGE-ERK1/2 signaling. Transgenic mice with diabetes that also overexpress RAGEs show renal enlargement, glomerular hypertrophy, mesangial expansion and advancing glomerulosclerosis, just as in humans [98]. We have noted that F2 isoprostanes promote TGF β synthesis in experimental diabetes [47]. It has been demonstrated that even short periods of hyperglycaemia are capable of inducing renal fibrosis [99].

Atherogenesis

Chronic renal failure is a harbinger of premature atherosclerosis in which LDL permeate the arterial walls. LDL is then oxidized by endothelial cells, macrophages and VSMs, in part by lipoxygenases [100]. Generally lipoproteins can be oxidized by (i) free and bound metal ions, (ii) ROS, (iii) peroxynitrite, (iv) myeloperoxidase, (v) lipoxygenases and (vi) L-cystine disulphide in VSMs. Of course NO affords protection but asymmetric dimethylarginine accumulates early in uremia and inhibits iNOS [92].

Oxidative signals are inducers of adhesion molecule expression on endothelial cells. The small GTP-binding protein Rac.1 is activated by various proinflammatory agents and it promotes superoxide generation via NADPH oxidases [101]. Rac.1 is involved too in the activation of NF- κ B and thus in formation of cytokines and chemokines, and expression of adhesion molecules. Thus

VCAM-1 is important for atherogenesis. Reactive aldehydes (carbonyls) play a critical role in the genesis of atherosclerosis [102]. Naturally HDL protects against the deleterious effects of oxidised LDL. HDL attenuates the expression of adhesion molecules, and it induces endothelial nitric oxide synthase and it thereby helps to promote vasorelaxation [103]. However, the myeloperoxidase product hypochlorous acid via 3-chlorotyrosine oxidises HDL in the arterial walls and so reverse cholesterol transport is impaired [104].

Chronic Renal Failure

Patients with chronic renal failure are in a state of 'oxidative stress' as shown by their elevated GSSG/GSH ratio, and the raised plasma lipid hydroperoxides and diene conjugates [105] and AOPPs [106]. In uraemia per se there is an accumulation of AGEs and of advanced lipoxidation end-products [107]. Undoubtedly there is enhanced oxidation of patient LDL before institution of haemodialysis (HD) [108]. F₂-isoprostanes are raised and that is not due to the dialysis process [109]. Plasma albumin is a target of the oxidative stress [110].

Once HD has started complement activation by the dialysis membranes provokes ROS formation by neutrophils and monocytes [106, 111]. Oxidatively modified proteins trigger the oxidative burst in neutrophils and monocytes [106, 112]. Neutrophils of HD patients exhibit higher spontaneous production of ROS [113]. The accumulation of mediators is proinflammatory [114, 115]. Production of chemokines is enhanced [116]. Such patients have a raised C-reactive protein [117] and are more liable to cardiovascular disease [118]. Nitrated proteins are elevated in the plasma [119]. A study of matrix metalloproteinase MMP-2 and its tissue inhibitors, TIMPs, in HD patients with and without cardiovascular disease showed elevated values, especially in patients with cardiovascular disease [120].

As Vaziri [121] has succinctly concluded, oxidative stress in patients with chronic renal failure is aggravated by hypertension, diabetes and autoimmune processes. For patients on dialysis there is the added burden of the dialysis therapy, parenteral iron and intercurrent infections. All this is clear, but aspects of the biochemistry still require clarification.

References

- Cooper CE, Patel RP, Brookes PS, Darley-Usmar VM: Nanotransducers in cellular redox signalling: Modification of thiols by reactive oxygen and nitrogen species. *Trends Biochem Sci* 2002;27:489–492.
- Soberman RJ: The expanding network of redox signalling. *J Clin Invest* 2003;111:571–574.
- Jones DP, Go Y-M, Anderson CL, Ziegler TR, Kincaid JM, Kirilov WG: Cysteine/cystine couple is a newly recognized biologic redox signalling control. *FASEB J* 2004;18:1246–1248.
- Dalle-Donne I, Giustarini D, Colombo R, Rossi R, Milzani A: Protein carbonylation in human diseases. *Trends Mol Med* 2003;9:169–175.
- Miyata T, van Ypersele de Strihou C, Kurokawa K, Baynes JW: Alterations in nonenzymatic biochemistry in uremia: Origin and significance of 'carbonyl stress' in long-term uremic complications. *Kidney Int* 1999;55:389–399.
- Marnett LJ, Riggins JN, West JD: Endogenous generation of reactive oxidants and electrophiles and their reactions with DNA and protein. *J Clin Invest* 2003;111:583–593.
- Csallany AS, Kim S-S, Gallaher DD: Response of urinary lipophilic aldehydes and related carbonyl compounds to factors that stimulate lipid peroxidation in vivo. *Lipids* 2000;35:855–862.
- Cambiaggi C, Dominici S, Comporti M, Pompella A: Modulation of human T lymphocyte proliferations by 4-hydroxynonenal, the bioactive product of neutrophil-dependent lipid peroxidation. *Life Sci* 1997;61:777–785.
- Locatelli F, Canaud B, Eckhardt K-U, Stenvinkel P, Wanner C, Zoccali C: Oxidative stress in end-stage renal disease: An emerging threat to patient outcome. *Nephrol Dial Transplant* 2003;18:1272–1280.
- Lawson JA, Rokach J, Fitzgerald GA: Isoprostanes: Formation, analysis and use as indices of lipid peroxidation in vivo. *J Biol Chem* 1999;274:24441–24444.
- Gamaley IA, Klyubin IV: Roles of reactive oxygen species: Signalling and regulation of cellular functions. *Int Rev Cytology* 1999;188:203–255.
- Dröge W: Free radicals in the physiological control of cell function. *Physiol Rev* 2002;82:47–95.
- Sowers JR: Hypertension, angiotensin II and oxidative stress. *N Engl J Med* 2002;346:1999–2001.
- Heitzer T, Schlinzig T, Krohn K, Meinertz T, Münzel T: Endothelial dysfunction, oxidative stress and risk of cardiovascular events in patients with coronary artery disease. *Circulation* 2001;103:1282–1288.
- Galle J, Wanner C: Oxidative stress and vascular injury – Relevant for atherogenesis in uraemic patients? *Nephrol Dial Transplant* 1997;12:2480–2483.
- Meister A: On the antioxidant effects of ascorbic acid and glutathione. *Biochem Pharmacol* 1992;44:1905–1915.
- Harris ED: Regulation of antioxidant enzyme. *FASEB J* 1992;6:2675–2683.
- Krinsky NI: Mechanism of action of biological antioxidants. *Proc Soc Exp Biol Med* 1992;200:248–254.
- Yu BP: Cellular defenses against damage from reactive oxygen species. *Physiol Rev* 1994;74:139–162.
- Hirota K, Murata M, Sachi Y, Nakamura H, Takeuchi J, Mori K, Yodoi J: Distinct roles of thioredoxin in the cytoplasm and in the nucleus: A two-step mechanism of redox regulation of transcription factor NF-kappaB. *J Biol Chem* 1999;274:27891–27897.
- Nguyen MT, Lue H, Kleemann R, Thiele M, Tolle G, Finkelmeier D, Wagner E, Braun A, Bernhagen J: The cytokine macrophage migration inhibitory factor reduces pro-oxidative stress-induced apoptosis. *J Immunol* 2003;170:3337–3347.
- Schopfer FJ, Baker PR, Freeman BA: NO-dependent protein nitration. *Trends Biochem Sci* 2003;28:646–654.

- 22 Pfeilschifter J, Eberhardt W, Beck KF, Huwiler A: Redox signaling in mesangial cells. *Nephron Exp Nephrol* 2003;93:e23–26.
- 23 Chiarugi P, Cirri P: Redox regulation of protein tyrosine phosphatases during receptor tyrosine kinase signal transduction. *Trends Biochem Sci* 2003;28:509–513.
- 24 Zhuang S, Schnellmann RG: H₂O₂-induced transactivation of EGF receptor requires Src and mediates ERK1/2 but not Akt activation in renal cells. *Am J Physiol Renal Physiol* 2004;286:F858–865.
- 25 Dalton TP, Shertzer HG, Puga AA: Regulation of gene expression by reactive oxygen. *Annu Rev Pharmacol Toxicol* 1999;39:67–99.
- 26 Klahr S, Morrissey J: Obstructive nephropathy and renal fibrosis. *Am J Physiol Renal Physiol* 2002;283:F861–F875.
- 27 Gooch JL, Gorin Y, Zhang B-X, Abboud HE: Involvement of calcineurin in transforming growth factor- β -mediated regulation of extracellular matrix accumulation. *J Biol Chem* 2004;279:15561–15570.
- 28 Sundaresan M, Yu ZX, Ferrens VJ, Irani K, Finkel T: Requirements for generation of H₂O₂ for platelet-derived growth factor signal transduction. *Science* 1995;270:296–299.
- 28b Bae YS, Sung J-Y, Lim C-S, Kim YJ, Hur KC, Kazlauskas A, Rhee SG: Platelet-derived growth factor-induced H₂O₂ production requires the activation of phosphatidylinositol 3-kinase. *J Biol Chem* 2000;275:10527–10531.
- 29 Wilmer WA, Tan LC, Dickerson JA, Danne M, Ravin BH: Interleukin-1 induction of MAP kinases in human mesangial cells: Role of oxidation. *J Biol Chem* 1997;272:10877–10881.
- 30 Radeke HH, Meier B, Turley N, Floege J, Hammermehl G, Resch G: Interleukin-1 and TNF α induce oxygen radical production in mesangial cells. *Kidney Int* 1990;37:747–751.
- 31 Satriano JA, Shuldinger M, Horq K, Xing Y, Shen Z, Schlondorff D: Oxygen radicals as second messengers for expression of the monocyte chemoattractant protein MCP-1 and colony stimulating factor CSF-1 in response to TNF α and immunoglobulin G. *J Clin Invest* 1993;92:1564–1571.
- 32 Oberle GP, Niemeyer J, Thaiss F, Schoeppe W, Stahl RAK: Increased oxygen radical and eicosanoid formation in immune-mediated mesangial injury. *Kidney Int* 1992;42:69–74.
- 33 Feng L, Xia Y, Garcia GE, Hwang D, Wilson CB: Involvement of reactive oxygen intermediates in cyclooxygenase-2 expression induced by interleukin-1, TNF α and lipopolysaccharide. *J Clin Invest* 1995;95:1669–1675.
- 34 Soberman RJ, Christmas P: The organization and consequences of eicosanoid signaling. *J Clin Invest* 2003;111:1107–1113.
- 35 Schreck R, Bauerle PA: A role for oxygen radicals as second messengers. *Trends Cell Biol* 1991;1:39–42.
- 36 Massy ZA, Guijarro CO, Donnell MP, Kim Y, Kashton CE, Egido J, Kasiske BL, Keane WF: The central role of nuclear factor- κ B in mesangial cell activation. *Kidney Int* 1991;71(suppl):S76–S79.
- 37 Anderson MT, Staal FJT, Gitler C, Herzenberg PA: Separation of oxidant-initiated and redox-regulated steps in the NF- κ B signal transduction pathways. *Proc Natl Acad Sci USA* 1994;91:11527–11531.
- 38 Khachigian LM, Collins T, Fries JWU: N-acetylcysteine blocks mesangial VCAM-1 and NF- κ B expression in vivo. *Am J Pathol* 1997;151:1225–1229.
- 39 Lander HM, Ogiste JS, Teng KK, Novogrodsky A: p21ras as a common signaling target of reactive free radicals and cellular redox stress. *J Biol Chem* 1995;270:21195–21140.
- 40 Lo YYC, Wong JMS, Cruz TF: Reactive oxygen species mediate cytokine activation of c-JUN NH₂ terminal kinases. *J Biol Chem* 1996;271:15703–15707.
- 41 Visarius TM, Putt DA, Schare JM, Pegouske DM, Lash LH: Pathways of glutathione metabolism and transport in isolated proximal tubular cells from rat kidney. *Biochem Pharmacol* 1996;52:259–272.
- 42 Liu B, Andrieu-Abadie N, Levade T, Zhang P, Obeid LM, Hannun YA: Glutathione regulation of neutral sphingomyelinase in tumor necrosis factor α -induced cell death. *J Biochem* 1998;273:11313–11320.
- 43 Veda N, Camargo SMR, Hang Y, Basmakian AG, Walker PD, Shah SV: Role of ceramide synthase in oxidative injury to renal tubular epithelial cells. *J Am Soc Nephrol* 2001;12:2384–2391.
- 44 Neale TJ, Ullrich R, Ojha P, Poczewski H, Verhoeven AJ, Kerjaschki D: Reactive oxygen species and neutrophil respiratory burst cytochrome b558 are produced by kidney glomerular cells in passive Heymann nephritis. *Proc Natl Acad Sci USA* 1993;90:3645–3649.
- 45 Jahngen-Hodge J, Obin MS, Gang Y, Shang F, Nowell TR, Gang J, Abai H, Blumberg JM, Taylor A: Regulation of ubiquitin conjugating enzymes by glutathione following oxidative stress. *J Biol Chem* 1997;272:28218–28226.
- 46 Bureau F, Desmet C, Melotte DA, Jaspar F, Volanti C, Vanderplassen A, Pastoret PP, Piette J, Lekeux P: A proinflammatory role for cyclopentenone prostaglandins at low micromolar concentrations: Oxidative stress-induced extracellular signal-regulated kinase activation without NF- κ B inhibition. *J Immunol* 2002;168:5318–5325.
- 47 Montero A, Munger KA, Khan RZ, Valdivielso JM, Morrow JD, Guash A, Ziyadeh F N, Badr KF: F2 isoprostanes mediate high glucose induced TGF- β synthesis and glomerular proteinuria in experimental type 1 diabetes. *Kidney Int* 2000;58:1967–1972.
- 48 Kyow M, Yoshizumi M, Tsuchiya K, Kirima K, Suzuki Y, Abe S, Hasegawa T, Tamaki T: Antioxidants inhibit ET-1(1–31) induced proliferation of vascular smooth muscle cells. *Biochem Pharmacol* 2002;64:1521–1531.
- 49 Ghibelli L, Coppola S, Fanelli C, Rotilio G, Civitareale P, Scovassi AI, Ciriolo MR: Glutathione depletion causes cytochrome-C release in the absence of commitment to apoptosis. *FASEB J* 1999;13:2031–2036.
- 50 Pias EK, Aw TY: Apoptosis is induced by cellular redox imbalance independent of ROS production. *FASEB J* 2002;16:781–790.
- 51 Kagan VE, Gleiss B, Tyurina YY, Tyurin VA, Elenstrom-Magnusson C, Liu S-X, Serinkan FB, Arroyo A, Chandra J, Orrenius S, Fadeel B: A role for oxidative stress in apoptosis: Oxidation and externalization of phosphatidylserine is required for macrophage clearance of cells undergoing Fas-mediated apoptosis. *J Immunol* 2002;169:487–499.
- 52 Gergely P Jr, Niland B, Gonchoroff N, Pullman R Jr, Phillips PE, Perl A: Persistent mitochondrial hyperpolarization, increased reactive oxygen intermediate production, and cytoplasmic alkalization characterize altered IL-10 signalling in patients with systemic lupus erythematosus. *J Immunol* 2002;169:1092–1101.
- 53 Verweij CL, Gringhuis SI: Oxidants and tyrosine phosphorylation: Role of acute and chronic oxidative stress in T and B lymphocyte signaling. *Antioxid Redox Signal* 2002;4:543–551.
- 54 Kawada N, Imai E, Karber A, Welch WJ, Wilcox CS: A mouse model of angiotensin II slow pressor response: Role of oxidative stress. *J Am Soc Nephrol* 2002;13:2860–2866.
- 55 Rathaus M, Bernheim J: Oxygen species in the microvascular environment: Regulation of vascular tone and the development of hypertension. *Nephrol Dial Transplant* 2002;17:216–221.
- 56 Cuzzocrea S, Mazzon E, Dugo L, DiPaola R, Caputi AP, Salvemini D: Superoxide: A key player in hypertension. *FASEB J* 2004;18:94–101.
- 57 Brisseau GF, Dachiw APB, Cheung PYC, Christie N, Rotstein OD: Posttranscriptional regulation of macrophage tissue factor expression by antioxidant. *Blood* 1995;85:1025–1035.
- 58 El Bekay R, Alvarez M, Monteseirin J, Alba G, Chacon P, Vega A, Martin-Nieto J, Jimenez J, Pintado E, Bedoya FJ, Sobrino F: Oxidative stress is a critical mediator of the angiotensin II signal in human neutrophils: Involvement of mitogen-activated protein kinase, calcineurin, and the transcription factor NF- κ B. *Blood* 2003;102:662–671.
- 59 Gonzalez-Pacheco FR, Caramelo C, Castilla MA, Deudero JJP, Arias J, Yague S, Jimenez S, Bragado R, Alvarez-Arroyo MV: Mechanism of vascular smooth muscle cell activation by hydrogen peroxide: Role of phospholipase-C. *Nephrol Dial Transplant* 2002;17:392–398.
- 60 Irani K: Oxidative signaling in vascular cell growth, death and survival: A review of the roles of reactive oxygen species in smooth muscle and endothelial cell mitogenic and apoptotic signaling. *Circ Res* 2000;87:179–183.
- 61 Bhastaran M, Reddy K, Radhakrishnan N, Franki N, Ding G, Singhal PC: Angiotensin II induces apoptosis in renal proximal tubular cells. *Am J Physiol Renal Physiol* 2003;284:F955–F965.

- 62 Giner V, Tormos C, Chaves FJ, Saez G, Redon J: Microalbuminuria and oxidative stress in essential hypertension. *J Intern Med* 2004;255: 588–594.
- 63 Ysebaert DK, De Greef KE, Vercauteren SR, Ghielli M, Verpooten GA, Eyskens EJ, De Broe ME: Identification and kinetics of leukocytes after severe ischaemia-reperfusion renal injury. *Nephrol Dial Transplant* 2000;15:1562–1574.
- 64 Granger DN, Hollwarth ME, Parks AD: Ischemia-reperfusion injury: Role of oxygen-derived free radicals. *Acta Physiol Scand* 1986;S548:47–63.
- 65 Molitoris BA, Marrs J: The role of cell adhesion molecules in ischemic acute renal failure. *Am J Med* 1999;106:583–592.
- 66 Daemen MA, van't Veer C, Denecker G, Heemskerk VH, Wolfs TG, Clauss M, Vandenameele P, Buurman WA: Inhibition of apoptosis induced by ischaemia-reperfusion prevents inflammation. *J Clin Invest* 1999;104: 541–549.
- 67 Alonso de Vega JM, Diaz J, Serrano E, Carbonell LF: Oxidative stress in critically ill patients with systemic inflammatory response syndrome. *Crit Care Med* 2002;30:1782–1786.
- 68 Shah SV: Role of reactive oxygen metabolites in experimental glomerular disease. *Kidney Int* 1989;385:1093–1106.
- 68b Shah SV: Oxidants and iron in chronic kidney disease. *Kidney Int Suppl* 2004; 91:S50–S55.
- 69 Budisavljevic MN, Hodge L, Barber K, Fulmer JR, Durazo-Arvizu RA, Self SE, Kuhlmann M, Raymond JR, Greene EL: Oxidative stress in the pathogenesis of experimental mesangial proliferative glomerulonephritis. *Am J Physiol Renal Physiol* 2003;285:F1138–F1148.
- 70 Kantengwa S, Jornot L, Devenoges C, Nicod LP: Superoxide anions induce the maturation of human dendritic cells. *Am J Respir Crit Care Med* 2003;167:431–437.
- 71 Marikovsky M, Ziv V, Nevo N, Harris-Ceruti C, Mahler O: Cu/Zn superoxide dismutase plays important role in immune response. *J Immunol* 2003;170:2993–2300.
- 72 Suzuki Y, Gomez-Guerrero C, Shirato I, Lopez-Franco O, Gallego-Delgado J, Lazaro A, Egido J: Pre-existing glomerular immune complexes induce polymorphonuclear cell recruitment through an Fc receptor-dependent respiratory burst: Potential role in the perpetuation of immune nephritis. *J Immunol* 2003;170: 3243–3253.
- 72b Descamps-Latscha B, Witko-Sarsat V, Nguyen-Khoa T, Nguyen AT, Gausson V, Mothu N, Cardoso C, Noel L-H, Guerin AP, London GM, Jungers P: Early prediction of IgA nephropathy progression: Proteinuria and AOPP are strong prognostic markers. *Kidney Int* 2004;66:1606–1612.
- 73 Trigwell SM, Radford PM, Page SR, Loweth AC, James RFL, Morgan NG, Todd I: Islet glutamic acid decarboxylase modified by reactive oxygen species is recognized by antibodies from patients with type 1 diabetes mellitus. *Clin Exp Immunol* 2001;126:242–249.
- 74 Ahsan H, Ali A, Ali R: Oxygen free radicals and systemic autoimmunity. *Clin Exp Immunol* 2003;131:398–404.
- 75 Schultz P, Lerner RA: From molecular diversity to catalysis: Lessons from the immune system. *Science* 1995;269:1835–1839.
- 76 Rodriguez-Iturbe B, Herrera-Acosta J, Johnson RJ: Interstitial inflammation, sodium retention and the pathogenesis of nephritic edema: A unifying hypothesis. *Kidney Int* 2002; 62:1379–1384.
- 77 Vaziri ND, Wang XQ, Oveisi F, Rad B: Induction of oxidative stress by glutathione depletion causes severe hypertension in normal rats. *Hypertension* 2000;36:142–146.
- 78 Rodriguez-Iturbe B, Vaziri ND, Herrera-Acosta J, Johnson RJ: Oxidative stress, renal infiltration of immune cells, and salt-sensitive hypertension: All for one and one for all. *Am J Physiol Renal Physiol* 2004;86:F606–F616.
- 79 Kanwar YS: Heme-oxygenase-1 in renal injury. *Kidney Int* 2001;59:378–379.
- 80 Lee TS, Chau LY: Heme oxygenase-1 mediates the anti-inflammatory effect of interleukin-10 in mice. *Nat Med* 2002;8:240–246.
- 81 Falk RJ, Jennette JC: ANCA are pathogenic. *J Am Soc Nephrol* 2002;13:1977–1979.
- 82 Gaut JP, Byun J, Tran HD, Loubser WM: Myeloperoxidase produces nitrating oxidants in vivo. *J Clin Invest* 2002;109:1311–1319.
- 83 Hampton MB, Vissers MCM, Keenan JI, Winterbourn CC: Oxidant mediated phosphatidylserine exposure and macrophage uptake of activated neutrophils: Possible impairment in chronic granulomatous disease. *J Leukoc Biol* 2002;71:775–781.
- 84 Vissers MCM, Lee W-G, Hampton MB: Regulation of apoptosis by vitamin C: Specific protection of the apoptotic machinery against exposure to chlorinated oxidants. *J Biol Chem* 2001;276:46385–46440.
- 85 Harper L, Ren Y, Savill J, Adu D, Savage C: Antineutrophil cytoplasmic antibodies induce reactive oxygen-dependent dysregulation of primed neutrophil apoptosis and clearance by macrophages. *Am J Pathol* 2000;157:211–220.
- 86 Cemerkeski S, Cantagrel A, van Meerwijk JPM, Romagnoli P: Reactive oxygen species differentially affect T cell receptor-signaling pathways. *J Biol Chem* 2002;272:19585–19593.
- 87 Tang S, Leung JCK, Abe K, Chan KW, Chan LYY, Chan TM, Lai KN: Albumin stimulates Interleukin-8 expression in proximal tubular epithelial cells in vitro and in vivo. *J Clin Invest* 2003;111:515–527.
- 88 Morigi M, Macconi D, Zojka C, Donadelli R, Buelli S, Zanchi C, Ghilardi M, Remuzzi G: Protein overload-induced NF- κ B activation in proximal tubular cells requires H₂O₂ through a PKC-dependent pathway. *J Am Soc Nephrol* 2002;13:1179–1189.
- 89 Iglesias J, Levine JS: Albuminuria and renal injury – Beware of proteins bearing gifts. *Nephrol Dial Transplant* 2001;16:215–218.
- 90 Zachwieja J, Zaniew M, Bobkowski W: Abnormal synthesis of cytokines in nephrotic syndrome – A consequence of increased oxidative stress (abstract M780)? *Nephrol Dial Transplant* 2003;18(suppl 4):251.
- 91 Brownlee M: Biochemistry and molecular cell biology of diabetic complications. *Nature* 2001;414:813–820.
- 91b Makita Z, Radoff S, Rayfield EJ, Yang Z, Skolnik E, Delaney V, Friedman EA, Cerami A, Vlassara: Advanced glycosylation end-products in patients with diabetic nephropathy. *N Engl J Med* 1991;325:836–842.
- 92 Horl WH: Atherosclerosis and uremic retention solutes. *Kidney Int* 2004;66:1719–1731.
- 93 Wautier M-P, Chappey O, Corda S, Stern DM, Schmidt AM, Wautier J-L: Activation of NADPH oxidase by AGEs links oxidative stress to altered gene expression via RAGE. *Am J Physiol Endocrinol Metab* 2001;280:E685–694.
- 94 Schmidt AM, Yan SD, Yan SF, Stern DM: The multiligand receptor RAGE as a progression factor amplifying immune and inflammatory responses. *J Clin Invest* 2001;108: 949–955.
- 95 Lal MA, Brismar H, Eklof A-C, Aperia A: Role of oxidative stress in advanced glycation end-product induced mesangial cell activation. *Kidney Int* 2002;61:2006–2014.
- 96 Kang BP, Frencher S, Reddy V, Kessler A, Malhotra A, Meggo LG: High glucose promotes mesangial cell apoptosis by oxidant dependent mechanism. *Am J Physiol Renal Physiol* 2003;53:F455–F466.
- 97 Kim Y, Kim BC, Song CY, Hing JK, Moon KC, Lee HS: Advanced glycosylation end-products stimulate collagen mRNA synthesis in mesangial cells mediated by protein kinase C and TGF β . *J Lab Clin Med* 2000;138:59–68.
- 98 Yamamoto Y, Kato I, Doi T, Yonekura H, Ohashi S, Takeuchi M, Watanabe T, Yamagishi S, Sakurai S, Takasawa S, Okamoto H, Yamamoto Y: Development and prevention of advanced diabetic nephropathy in RAGE-overexpressing mice. *J Clin Invest* 2001;108: 261–268.
- 99 Polhill TS, Saad S, Poronnik P, Fulcher GR, Pollock CA: Short-term peaks in glucose promote renal fibrogenesis independently of total glucose exposure. *Am J Physiol Renal Physiol* 2004;287:F268–F273.
- 100 Stocker R, Keaney JF: Role of oxidative modifications in atherosclerosis. *Physiol Rev* 2004;84:1381–1478.
- 101 Chen X-L, Zhang Q, Zhao R, Ding X, Tummala PE, Medford RM: Rac.1 and superoxide are required for the expression of cell adhesion molecules induced by TNF α in endothelial cells. *J Pharmacol Exp Ther* 2003; 305:573–580.
- 102 Himmelfarb J, Stenvinkel P, Ikizler TA, Hakim RM: The elephant in uremia: Oxidant stress as a unifying concept of cardiovascular disease in uremia. *Kidney Int* 2002;62:1524–1538.

- 103 Assmann G, Nofer JR: Atheroprotective effects of high density lipoproteins. *Ann Rev Med* 2003;54:321–341.
- 104 Bergt C, Pannathur S, Fu X, Byun JO, Brien K, McDonald TO, Singh P, Anantharamaiah GM, Chait A, Brunzell J, Geary RL, Oram JF, Heinecke JW: The myeloperoxidase product hypochlorous acid oxidizes HDL in the human artery wall and impairs ABCA1-dependent cholesterol transport. *Proc Natl Acad Sci USA* 2004;101:13032–13037.
- 105 Annuk M, Fellstrom B, Akerblom O, Zilmer K, Vihalemm T, Zilmer M: Oxidative stress markers in pre-uremic patients. *Clin Nephrol* 2001;56:308–314.
- 106 Witko-Sarsat V, Gausson V, Nguyen A-T, Touam M, Druke T, Santangelo F, Descamps-Latscha B: AOPP-induced activation of human neutrophil and monocyte oxidative metabolism: A potential target for N-acetylcysteine treatment in dialysis patients. *Kidney Int* 2003;64:82–91.
- 107 Miyata T, Sugiyama S, Saito A, Kurokawa K: Reactive carbonyl compounds related uremic toxicity (carbonyl stress). *Kidney Int* 2001 (suppl 178):S25–S31.
- 108 Diepeveen SHA, Verhoeven GHWE, van der Polen J, Dikkeschei BLD, van Tits LJ, Kolsters G, Offerman JJG, Bilo HJG, Stalenhoef AFH: Oxidative stress in patients with end-stage renal disease prior to the start of renal replacement therapy. *Nephron Clin Pract* 2004;98:c3–c7.
- 109 Ikizler TA, Morrow JD, Roberts J, Evanson JA, Becker B, Hakim RM, Shyr Y, Himmelfarb J: Plasma F2-isoprostane levels are elevated in chronic haemodialysis patients. *Clin Nephrol* 2002;58:190–197.
- 110 Himmelfarb J, McMonagle E: Albumin is the major plasma protein target of oxidant stress in uremia. *Kidney Int* 2001;60:358–363.
- 111 Kormocz GF, Wolfel UM, Rosenkranz AR, Horl WH, Oberbauer R, Zlabinger GJ: Serum protein modified by neutrophil-derived oxidants as mediators of neutrophil stimulation. *J Immunol* 2001;167:451–460.
- 112 Jacobs AA Jr, Ward RA, Welhausen SR, McLeish KR: Polymorphonuclear leukocyte function during hemodialysis: Relation to complement activation. *Nephron* 1989;52:119–124.
- 113 Himmelfarb J, Lazarus JM, Hakim R: Reactive oxygen species production by monocytes and polymorphonuclear leukocytes during dialysis. *Am J Kidney Dis* 1991;17:271–276.
- 114 Amore A, Coppo R: Immunological basis of inflammation in dialysis. *Nephrol Dial Transplant* 2002;17(suppl 8):16–24.
- 115 Schindler R: Microinflammation in renal failure. *Nephrol Dial Transplant* 2004;19(suppl 5):34–40.
- 116 Pawlak K, Pawlak D, Mysliwiec M: Oxidative stress influences CC-chemokine levels in hemodialyzed patients. *Nephron Physiol* 2004;96:p105–p112.
- 117 Spittle MA, Hoenich NA, Handelman GJ, Adhikarla R, Homel P, Levin NW: Oxidative stress and inflammation in haemodialysis patients. *Am J Kidney Dis* 2001;38:1408–1413.
- 118 Boaz M, Matas Z, Biro A, Katzir Z, Green M, Fainaru M, Smetana S: Serum malondialdehyde and prevalent cardiovascular disease in hemodialysis. *Kidney Int* 1999;56:1078–1083.
- 119 Mitrogianni Z, Barbouti A, Galaris D, Siamopoulos KC: Tyrosine nitration in plasma proteins from patients undergoing hemodialysis. *Am J Kidney Dis* 2004;44:286–292.
- 120 Pawlak K, Pawlak D, Mysliwiec M: Extrinsic coagulation pathway activation and metalloproteinase2/TIMPs system are related to oxidative stress and atherosclerosis in hemodialysis patients. *Thromb Haemost* 2004;92:646–653.
- 121 Vaziri ND: Oxidative stress in uremia: Nature, mechanisms and potential consequences. *Semin Nephrol* 2004;25:469–473.