Chronic kidney disease (CKD) is a common and serious problem that adversely affects human health, limits longevity and increases costs to health-care systems worldwide. Its increasing incidence cannot be fully explained by traditional risk factors. Oxidative stress is prevalent in CKD patients and is considered to be an important pathogenic mechanism. Oxidative stress develops from an imbalance between free radical production often increased through dysfunctional mitochondria formed with increasing age, type 2 diabetes mellitus, inflammation, and reduced anti-oxidant defences. Perturbations in cellular oxidant handling influence downstream cellular signalling and, in the kidney, promote renal cell apoptosis and senescence, decreased regenerative ability of cells, and fibrosis. These factors have a stochastic deleterious effect on kidney function. The majority of studies investigating anti-oxidant treatments in CKD patients show a reduction in oxidative stress and many show improved renal function. Despite heterogeneity in the oxidative stress levels in the CKD population, there has been little effort to measure patient oxidative stress levels before the use of any anti-oxidants therapies to optimize outcome. This review describes the development of oxidative stress, how it can be measured, the involvement of mitochondrial dysfunction and the molecular pathways that are altered, the role of oxidative stress in CKD pathogenesis and an update on the amelioration of CKD using anti-oxidant therapies.

One of the key functions of the kidneys is to filter waste products that build up in the blood. Renal failure determines that waste products are not removed completely or sufficiently. This can occur quickly (acute renal failure, or acute kidney injury) often as the result of ischaemia, toxins or mechanical trauma. More often, however, the development of renal failure is gradual and insidious, with resultant chronic kidney disease (CKD). It is often many years before noticeable loss of renal function occurs. People with CKD have a high risk of death from stroke or heart attack, and CKD may also progress to total and permanent renal failure (end-stage renal disease). Dialysis or transplantation is then necessary, with loss of quality of life, decreased individual life expectancy and increased costs to health-care systems. This review article focuses mainly on patients developing CKD.

Chronic kidney disease has increasing incidence and prevalence in developed and developing nations. The kidneys show the greatest age-associated chronic pathology compared with brain, liver and heart, and one in six adults over 25 years of age has some degree of CKD, with incidence increasing with age. A study of almost 20 000 ethnic Chinese men and women greater than 20 years of age demonstrated that changes in renal function could predict longevity. The structural characteristics of CKD include increased tubular atrophy, interstitial fibrosis, glomerulosclerosis, renal vasculopathy and reduced renal regenerative capability. These characteristics may be caused, at least in part, by the gradual loss of renal energy through development of mitochondrial dysfunction and resultant, increasing oxidative stress.

OXIDATIVE STRESS AND NORMAL KIDNEY METABOLISM

Oxidative stress may be defined as a disturbance in regular cellular and molecular function caused by an imbalance between production of reactive species and the natural anti-oxidant ability of our cells. Reactive oxygen species (ROS) and reactive nitrogen species often act together to create a state of oxidative stress. ROS are arguably the most
Table 1 Common physiological reactive species

<table>
<thead>
<tr>
<th>Reactive oxygen species</th>
<th>Free radicals†</th>
<th>Non-radicals‡</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide (O₂⁻)</td>
<td>Hydrogen peroxide (H₂O₂)</td>
<td></td>
</tr>
<tr>
<td>Hydroxyl (OH⁻)</td>
<td>Organic peroxides (R-OOH) e.g. lipid peroxides</td>
<td></td>
</tr>
<tr>
<td>Peroxyl (R-O⁻)</td>
<td>Hypochlorous acid (HOCI)</td>
<td></td>
</tr>
</tbody>
</table>

†A free radical is defined as any molecule that has an unpaired electron in its outer shell. ‡A non-radical does not contain unpaired electrons but is either an oxidizing agent or is easily converted to a free radical.

Table 2 Endogenous and exogenous sources of reactive species

<table>
<thead>
<tr>
<th>Endogenous factors</th>
<th>Exogenous factors</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mitochondrial oxidative phosphorylation</td>
<td>Environmental pollutants and toxins</td>
</tr>
<tr>
<td>Xanthine oxidase, NADPH oxidase</td>
<td>Cigarette smoke</td>
</tr>
<tr>
<td>Inflammatory cells</td>
<td>Hypoxia</td>
</tr>
<tr>
<td>Endosome/lysosome degradation</td>
<td>Radiation (sunlight, UV radiation)</td>
</tr>
<tr>
<td>Angiotensin II</td>
<td>Hypoxia</td>
</tr>
<tr>
<td>Fibroblasts</td>
<td>Infectious microbes</td>
</tr>
<tr>
<td>Endoplasmic reticulum</td>
<td>Glucotoxicity</td>
</tr>
<tr>
<td>Peroxisomes</td>
<td></td>
</tr>
</tbody>
</table>

Important of the free radicals in biological systems. A list of the common reactive species is found in Table 1. The main ROS are superoxide (O₂⁻), the hydroxyl radical (OH⁻) and hydrogen peroxide (H₂O₂). Examples of the endogenous and exogenous sources of reactive species are listed in Table 2.

Estimated levels of ROS within mitochondria are 5- to 10-fold higher than other cytosolic and nuclear compartments. Other contributing sites of ROS generation include the endoplasmic reticulum, peroxisomes and lysosomes. One to three per cent of inspired molecular oxygen is converted to O₂⁻, which is the most common of the ROS and a powerful precursor of H₂O₂. Although cellular H₂O₂ is stable, it has the potential to interact with a variety of substrates to cause damage, especially in the presence of the reduced metal ion Fe²⁺. This leads to H₂O₂ to break down and form the most reactive and damaging of the ROS, OH⁻. In healthy cells, the production of the potentially harmful H₂O₂ is countered by the catalysing actions of mitochondrial or cytosolic catalase (CAT) or thiol peroxidases into H₂O and O₂. Figure 1 demonstrates pathways to, and natural anti-oxidant neutralization of, common ROS.

Given that ROS are likely to be highly damaging molecules to cells, why have the mitochondria not evolved more efficient systems that limit mitochondrial oxidants? One possible answer is that ROS have an essential role in oxidant metabolism where they are involved in highly conserved basic physiological processes as effectors of downstream pathways. Thus, to some, oxidative stress theories of disease pathogenesis must be intrinsically flawed. Nonetheless, ROS are damaging molecules. Even when they are produced during normal respiration, they could cause cumulative damage that would eventually lead to loss of cell and tissue function and, ultimately, disease. Their production is known to increase, over natural anti-oxidant levels, during progressive disease and during ageing.

The kidney is highly energetic and therefore relies heavily on aerobic metabolism for the production of ATP by oxidative phosphorylation. The reduction of molecular O₂ along the electron transport chain (ETC) within mitochondria is vital for renal cellular function, yet potentially devastating long-term. The ETC consists of five multi-enzyme complexes responsible for maintaining mitochondrial membrane potential and ATP generation. Each of these complexes presents a site of ROS generation; however, complexes I and III have been identified as primary sites of O₂⁻ generation. Complex I, also known as nicotinamide adenine dinucleotide (NADH) dehydrogenase, or NADH-CoenzymeQ (NADH-CoQ) reductase, facilitates the transfer of electrons between NADH and CoQ10 (sometimes known as ubiquinone). Defects in oxidative phosphorylation may be due to the use of substrates in the respiratory chain, such as the reduced NADH and NADH oxidase, and not due to alterations in the proteins of the respiratory complexes. Thus, it is likely that altered respiratory complexes and substrates lead to an inefficiency of electron transport, and subsequent increased ROS, decreased ATP and a loss of the mitochondrial membrane potential. Oxidatively damaged proteins of the mitochondrial complexes increase with age in mice. In CKD patients (stages 2–3) and haemodialysis patients, impaired mitochondrial respiration was recorded. In this latter report, Granata and colleagues used a combined strategy based on high throughput genome-based microarray and classical molecular methodologies, to investigate the mechanisms underlying alterations in cell metabolism in CKD patients. In particular, oxidative phosphorylation system components were analysed. The results demonstrated clear deregulation of the mitochondrial respiratory machinery in CKD patients, closely associated with enhanced oxidative stress. These results may help explain other reports on CKD patients that indicate a subnormal energy metabolism in this population.

**NATURAL DEFENCES: ENDOGENOUS ANTI-OXIDANTS**

The production of ROS is usually in balance with the availability and cellular localization of anti-oxidant enzymes and thiols, such as superoxide dismutase (SOD), CAT, glutathione peroxidase (Gpx) and glutathione (GSH) (Fig. 2). GSH synthesis is dependent on ATP but the maintenance of its reducing power is dependent on NADPH and the pentose phosphate pathway. In vivo studies have found accumulated oxidative damage occurs from decreased levels of these endogenous anti-oxidants rather than increased ROS pro-
However, adequate levels of both are likely to be vital for normal cell function. Mitochondria possess their own pool of anti-oxidants such as mitochondrial manganese-SOD (Mn-SOD) to counteract their generation of ROS. Mn-SOD or copper/zinc-SOD (Cu/Zn-SOD) converts O$_2^-$ to H$_2$O$_2$, which is then decomposed to H$_2$O and O$_2$ by CAT and Gpx. Cu/Zn-SOD has been implicated in stabilizing O$_2^-$ within other cellular compartments, especially peroxisomes, and must be considered in maintenance of the redox state of the whole cell. Limited anti-oxidant actions of Cu/Zn-SOD may also occur within the inter-membrane space of the mitochondria.

Fig. 1 Cellular sites of free radical generation and interactions. Mitochondria produce high levels of the superoxide anion (O$_2^-$) as a by-product of oxidative phosphorylation. This is converted to the stable hydrogen peroxide (H$_2$O$_2$) by manganese superoxide dismutase (MnSOD). Protein assemblage by the endoplasmic reticulum and fatty acid breakdown by peroxisomes also account for O$_2^-$ generation, whereby conversion to H$_2$O$_2$ occurs due to copper/zinc superoxide dismutase (Cu/ZnSOD). Catalase (CAT) and glutathione peroxidase (Gpx) are able to neutralize H$_2$O$_2$ into water (H$_2$O) and oxygen (O$_2$). However, in the presence of high amounts of H$_2$O$_2$, heavy metals (Fe$^{3+}$ or Cu$^{2+}$) can generate the highly reactive hydroxyl anion (OH$^-$). Nitric oxide (NO$^-$) and O$_2^-$ have the ability to form the highly reactive nitrogen species, peroxynitrite (ONOO$^-$), which in the presence of carbon dioxide (CO$_2$) forms nitrogen dioxide (NO$_2$) and the reactive carbonyl species, the carboxyl anion (CO$_3^-$).

Fig. 2 Cellular sites of reducing oxidative stress by oxidant modifying compounds. Inflammation, lipid peroxidation and reactive oxygen species (ROS) from mitochondrial, cytoplasmic and extracellular sources contribute to oxidative stress. Vitamin E incorporates into the phospholipid bilayer of the cell membrane halting lipid peroxidation chain reactions. ω-3 fatty acids displace arachidonic acid in the cell membrane and thus reduce arachidonic-derived ROS, but also significantly reduce inflammation and subsequent fibrosis. The cysteine residue of N-acetyl-cysteine (NAC) is a precursor for glutathione (GSH) synthesis, and the thiol group is able to scavenge ROS directly. Allopurinol inhibits xanthine oxidase derived ROS and the damaging effects of uraemia. Coenzyme Q$_{10}$ (CoQ$_{10}$) enhances the efficiency of electron transport in the mitochondria, thereby reducing mitochondrial derived ROS, and is also able to directly scavenge ROS. Mitochondrial (mito)-targeted anti-oxidants accumulate in the mitochondria, reducing mitochondrial-derived ROS.
Among the various endogenous defences against ROS, glutathione homeostasis is critical for a cellular redox environment. Glutathione-linked enzymatic defences include Gpx, glutathione-S-transferase (GST), glutaredoxins (Grx), thioredoxins (Trx) and peroxiredoxins (Prx). Many of these proteins are known to interact with each other, forming networks that may be prone to dysfunction. Mitochondrial-specific isoforms of these proteins also exist, and these may be more critical for cell survival compared with their cytosolic counterparts. Mitochondrial dysfunction, resulting in depleted ATP synthesis, has the potential to reduce the redox control of glutathione because the rate of glutathione synthesis is ATP-dependent. In the kidney, intracellular synthesis of glutathione from amino acid derivatives (glycine, glutamate and cysteine) accounts for the majority of cellular glutathione compared with, for example, the uptake of extracellular glutathione from the basolateral membrane in epithelial tubular cells of the renal nephron. This characteristic may account for different segments of the nephron being differentially susceptible to the same insult, as proximal tubular epithelial cells lack the ability to synthesize glutathione, compared with other segments of the nephron, which have that ability.

**DYSFUNCTIONAL MITOCHONDRIA AND OXIDATIVE STRESS**

Mitochondrial biogenesis and degradation (mitophagy) usually occur in balance within healthy cells, and their imbalance may be a major contributor to oxidative stress and cellular metabolic decline. Mitophagy is carried out by autophagy, a process that was originally thought to be a non-selective cell regulatory mechanism for the degradation of dysfunctional organelles within the cellular lysosome system. More recently, the discovery of the autophagy (Atg) genes has uncovered a highly selective process for removal of damaged mitochondria. In particular, the mitochondrial transmembrane receptor gene Atg32 directs autophagosome formation. This response is enhanced by a decrease in ATP production due to dysfunctional mitochondria, and is regulated by the intracellular energy sensor, adenosine monophosphate-activated protein kinase. Should ATP reach critical levels through removal of too many dysfunctional mitochondria, autophagic cell death will be induced. Increasing mitochondrial biogenesis is an attractive target to reduce cellular metabolic injury. However, increasing the number of mitochondria could possibly worsen or induce tissue hypoxia due to increased oxygen consumption.

Oxidative stress also induces apoptosis, a process central to functional tissue loss in CKD. Oxidative stress-induced mitochondrial dysfunction and ROS generation may cause suppression of phosphorylation of the anti-apoptotic B-cell lymphoma-2 (Bcl-2) protein and loss of mitochondrial membrane potential. The intrinsic, mitochondrial-driven, pathway to apoptosis is of particular importance to age-related CKD. Opening of the mitochondrial permeability transition pore releases the pro-apoptotic factor cytochrome C (CytC). CytC is bound to the inner mitochondrial membrane by an association with the anionic phospholipid, cardiolipin. Increased ROS result in dissociation of CytC from cardiolipin, and increased amounts of CytC in the cytosol. Pro-apoptotic proteases, known as caspases, also play essential roles in apoptosis. Cytoplasmic CytC forms an apoptosome with apoptotic peptidase activating factor-1 and caspase-9, leading to cleavage and activation of caspase-9 and caspase-3, and the structural changes of apoptosis. The translocation of the Bcl-2 family proteins, especially pro-apoptotic Bax (Bcl-2-associated x protein) and Bak (Bcl-2 antagonist killer), to the mitochondria of kidney cells is the precursor to opening of the mitochondrial permeability transition pore, release of CytC and resultant apoptosis. These proteins can interact with the outer mitochondrial membrane, causing its permeabilization. Endogenous anti-apoptotic Bcl-XL (the Bcl-X long isoform) also translocates from the cytoplasm to the mitochondrial membrane, and is known to protect renal distal tubular epithelium against oxidative stress.

**OXIDATIVE STRESS IN CKD**

During the pathogenesis of CKD, perturbations in cellular oxidant handling influence downstream cell signalling and, in the kidney, promote renal cell apoptosis and senescence, decreased regenerative ability of cells and fibrosis. The pathways are tightly controlled, with transcription often determined by specific transcription factors, and post-translational modifications that include phosphorylation, methylation, acetylation, ubiquitination and O-GlcNAcylation to regulate outcomes. Several of these genes, which are regulated by oxidative stress and may act in the development of CKD, are reviewed in the following paragraph.

The Forkhead (FoxO) proteins are a family of transcription factors that play a critical role in the regulation of genes in ageing. They comprise FoxO1 to FoxO4 and FoxO6; however, FoxO1 has most association with CKD. FoxO1 has increased levels of phosphorylation in the kidneys of elderly overweight people with type 2 diabetes and CKD and old hypertensive rats with CKD. FoxOs induce apoptosis mainly by upregulation of pro-apoptotic genes such as Bax, yet they can also detoxify harmful cellular oxidants like O₂⁻ and H₂O₂ and protect cells. Their exact role in oxidative stress-induced CKD needs further investigation. Nuclear factor-kappa B (NF-kB) comprises a family of rapid-acting nuclear transcription factors that transcriptionally regulate a wide variety of genes involved in inflammation, immunity, apoptosis, cell proliferation and differentiation. In oxidative stress-induced kidney disease, NF-kB is activated by ROS and initiates signalling pathways involved in renal fibrosis. It has been implicated in the transcriptional activation of the...
cell cycle inhibitor p21, linking this transcriptional regulator with renal cell senescence. The adapter protein p66<sup>shc</sup> is a mediator of mitochondrial dysfunction. An isoform of the ShcA protein, p66<sup>shc</sup> antagonizes the cell proliferative actions of two other isoforms, p46<sup>shc</sup> and p52<sup>shc</sup>. Oxidative stress induces the phosphorylation of serine 36 of p66<sup>shc</sup> before its translocation into the mitochondria. Here, it translates oxidation of cysteine residues. Although the role of p66<sup>shc</sup> has been noted in glomerulopathies and diabetes, and its differential expression has been demonstrated in ageing kidneys, the functional significance of p66<sup>shc</sup> in the pathogenesis of CKD needs further investigation.

Uremic toxins may also be a source of oxidative stress in CKD patients. Uric acid is the hepatic end-product of purine metabolism in humans. It is synthesized by xanthine oxidoreductase, which catalyses the oxidation of hypoxanthine to xanthine and xanthine to uric acid. Resulting hyperuricaemia is associated with an increased risk for developing CKD and enhances its progression. In addition, retention of uremic toxins promotes inflammation, and therefore oxidative stress, by priming polymorphonuclear lymphocytes, activating IL-1β and IL-8 and stimulating the innate immune response through CD8<sup>+</sup> cells. Uric acid synthesis can also promote oxidative stress directly through the activity of xanthine oxidoreductase. This enzyme is synthesized as xanthine dehydrogenase, which can be converted to xanthine oxidase by calcium-dependant proteolysis or modification of cysteine residues. In doing so, the enzyme loses its capacity to bind NADH by alterations in its catalytic site and, instead, transfers electrons to O<sub>2</sub>, thereby generating O<sub>2</sub><sup>-</sup>. However, the role of uric acid in many conditions associated with oxidative stress is not clear and there are experimental and clinical data showing that uric acid also has a role in vivo as an anti-oxidant.

**MEASURING OXIDATIVE STRESS**

Free radicals have extremely short half-lives, so that in most cases oxidative stress is measured by specific end-products of the process. Reactive species can be measured directly by electron paramagnetic resonance or various spin trapping methods, but these methods present some practical limitations, especially in humans. At present, they are costly, and their safety and efficacy have not been proven. Oxidative stress biomarkers are available, and it is their use that has indicated a positive correlation between increasing oxidative stress with increasing stages of CKD. Assays for oxidative stress or anti-oxidant status and some of the popular biomarkers are shown in Table 3, which also indicates whether the end-product can be measured in urine, serum, tissue, cell culture or other biological products. Common and reliable assays for oxidative stress in CKD in humans are discussed specifically.

**Isoprostanes**

As with most oxidative stress biomarkers, the isoprostanes detect levels of specific end-products from free radical damage. They are considered by some researchers to be the best available biomarker of lipid peroxidation and have been investigated in the pathogenesis of CKD. Studies have focused primarily on F<sub>2</sub>-isoprostanes, which are formed by non-enzymatic peroxidation of arachidonyl lipids. Specifically, 8-isoprostane (8-epi-PGF<sub>2a</sub>) is measured. F<sub>2</sub>-isoprostanes are best detected using mass spectroscopy, and urine and plasma are typically used. One of their limitations as a biomarker of oxidative stress is that they are rapidly metabolized and, as a result, any increase in plasma isoprostane concentration may be due not only to their increased formation from lipid peroxidation, but also to a slower metabolism. Measurements of F<sub>2</sub>-isoprostanes also have relatively low reproductibility, for example, in the one healthy patient on a defined diet and exercise regimen, carried out at the same time of day on subsequent days. A final, important, consideration is that the F<sub>2</sub>-isoprostanes, like all end-product biomarkers, are a measure of whole-body oxidative stress rather than oxidative stress localized only to the kidney. Nevertheless, the use of isoprostanes has delivered important information on increased oxidative stress and related loss of kidney function, early in the progression of CKD.

**Malondialdehyde**

Malondialdehyde (MDA) is another end-product generated by lipid peroxidation and has been used to demonstrate increased oxidative stress during CKD. Unlike F<sub>2</sub>-isoprostanes, MDA has the ability to react further and possibly cause protein and DNA adducts, thus levels of MDA should be interpreted with caution. MDA, along with other lipid peroxidation products such as 4-hydroxynonenals, is a thiobarbituric acid reactive substance (TBARS). Earlier investigations into oxidative stress commonly assayed TBARS; however, simple TBARS assays are unreliable measures of oxidative stress because most TBARS in human body fluids are formed non-specifically and artfactually, and are not specifically related to lipid peroxidation. High-performance liquid chromatography extraction of MDA from plasma, with subsequent quantification, is considered a reliable measure of oxidative stress. Improved methods derivatize MDA with 2,4-dinitrophenylhydrazine, which forms specific hydrazones for MDA that can be separated by high-performance liquid chromatography and quantified using methyl-MDA as an internal standard. Urinary MDA as a measure of impaired kidney function in patients can be difficult to interpret given that renal clearance of MDA possibly provides an adaptive mechanism to prevent lipid peroxidation accumulating within kidney tubular cells.
Other common assays or biomarkers of oxidative stress in CKD patients

Advanced oxidation protein products (AOPP) accumulate in the serum of CKD patients, especially those with uraemia and diabetes, contributing to the pathogenesis of CKD. AOPP are primarily derived from serum albumin following hypochlorous acid free radical attack and they provide a valuable indicator of oxidation-mediated protein damage. The prevalence of albuminuria/proteinuria in CKD and its impact on AOPP has not yet been investigated. Protein carbonyl assays quantify the carbonyl groups associated with oxidant-damaged proteins. Protein carbonyls are not specific for oxidative stress as they also measure glycated proteins and bound aldehydes. An increase in protein carbonyls was demonstrated in CKD patients in stages 3–5, yet no correlation was found between protein carbonyl levels and decreased GFR. The pathogenesis of type 2 diabetes includes oxidative stress as a mechanism. Protein carbonyls are increased in plasma and lymphocytes of diabetes patients compared with healthy control. γ-Glutamyl transpeptidase (GGT) has been trialled as a biomarker of CKD onset through the mechanism of oxidative stress. Extracellular GGT is required to metabolize extracellular-reduced glutathione, allowing for the intracellular synthesis of glutathione. Serum anti-oxidant levels had an inverse relationship to serum GGT, indicating a redox-regulating role. The relationship between plasma and extracellular GGT is not fully defined, but it does appear that serum GGT presents a favourable biomarker of oxidative stress. It is stable, quick and inexpensive to test, and provides an indication of whole-body oxidative stress compared with specific oxidative damage to lipids, DNA or proteins. In an excellent review of measures of oxidative stress, Halliwell and colleagues discuss more broadly the different measures of oxidative stress, including reasons leading to poor correspondence between markers, like the rapid metabolism of isoprostanes compared with the slower metabolism of oxidized proteins.

COMBATING CKD BY TARGETING OXIDATIVE STRESS

Two major goals for controlling development of CKD are early detection and slowing progression to end-stage renal
Oxidative stress and CKD

Vitamin E

Vitamin E comprises a family of eight different lipid-soluble tocophersols and tocotrienols that scavenge free radicals by incorporating into the plasma membrane of cells, thus halting lipid peroxidation chain reactions. Vitamin E food-stuffs primarily consist of \( \alpha \)-tocotrienol, which has a higher anti-oxidant efficacy; however, \( \alpha \)-tocopherol has higher bio-availability \textit{in vivo} than the other seven compounds and so the focus has been on its usage. The basis of vitamin E supplementation is to enhance \( \alpha \)-tocopherol levels in cell plasma membranes to prevent lipid peroxidation and resultant oxidative stress. Vitamin E is often delivered with vitamin C in an attempt to boost the anti-oxidant efficacy, as vitamin C has been shown to assist in recycling vitamin E. One drawback of \( \alpha \)-tocopherol is that it takes several days of pretreatment to exhibit anti-oxidant effects.\(^{58}\) Trolox (\( \alpha \)-hydroxy-2,5,7,8-tetramethylchromane-2-carboxylic acid), is an analogue of \( \alpha \)-tocopherol and has shown far better free radical scavenging properties owing to its water solubility. The majority of \textit{in vivo} studies using Trolox have reported beneficial effects in acute cases of renal injury such as ischaemia reperfusion, due to rapid solubility and increased potency.\(^{39}\) A combination supplement containing both \( \alpha \)-tocopherol and Trolox may offer greater efficacy due to the fast-acting activities of Trolox combined with the sustained scavenging actions of \( \alpha \)-tocopherol.

Patients with progressive CKD display the largest decrease in serum \( \alpha \)-tocopherol levels, indicating an increased need for \( \alpha \)-tocopherol in the CKD population.\(^{37}\) Supplementation of \( \alpha \)-tocopherol in an end-stage kidney disease dialysis population reduced the risk of associated cardiovascular disease, decreased oxidative stress and increased erythrocyte anti-oxidants SOD, Gpx and CAT.\(^{60}\) However, in a meta-analysis by Miller and colleagues,\(^{61}\) based on the combination of several studies, an increase in all-cause mortality was found with high-dose vitamin E (\( \geq 400 \) IU/day) in patients with chronic diseases.\(^{62}\) Furthermore, the SELECT trial demonstrated that dietary supplementation with vitamin E significantly increased the risk of prostate cancer among healthy men.\(^{63}\) Future trials should determine the cause of these risks as well as focus on \( \gamma \)- and \( \delta \)-tocopherol supplementation.

Omega-3 polyunsaturated fatty acids

Although considered more an anti-inflammatory\(^{64}\) than anti-oxidant treatment, long chain omega(\( \omega \))-3 polyunsaturated fatty acids, including docosahexanoic acid and eicosapentaenoic acid, have been investigated in a large range of \textit{in vitro} and \textit{in vivo} CKD models. They were found to enhance endogenous anti-oxidant defence systems such as \( \gamma \)-glutamyl-cysteinyl ligase and glutathione reductase.\(^{65}\) In models of progressive renal fibrosis, kidney function and structure were improved using eicosapentaenoic acid and docosahexanoic acid supplementation, with reduced oxidative stress, inflammation and tubulointerstitial fibrosis.\(^{66}\) Use of \( \omega \)-3 polyunsaturated fatty acids in human CKD patients is under multicentre trials and the anti-oxidant status of the patients will, hopefully, be recorded in these trials.

N-acetyl cysteine

N-acetyl cysteine (NAC) is an essential precursor to many endogenous anti-oxidants involved in the decomposition of peroxides. It attenuates oxidative stress from various underlying causes by replenishing intracellular glutathione stores. The limiting precursor to glutathione biosynthesis is L-cysteine. This amino acid is not readily available in the human diet and this was the primary basis for NAC supplementation – to replenish cysteine levels. However, the sulphhydryl-thiol group of L-cysteine is also able to exert direct anti-oxidant effects by scavenging free radicals. The results of NAC supplementation in kidney disease have been variable. NAC pretreatment reduced endothelial dysfunction caused by uremic toxins by reducing ROS-dependent expression of NF-\( \kappa \)B.\(^{67}\) NAC reduced kidney MDA levels in a mouse model of diabetic nephropathy.\(^{68}\) The treatment of CKD patients with NAC has been largely disappointing,\(^{69}\) but in end-stage kidney disease patients receiving either haemodialysis or peritoneal dialysis, NAC reduced serum 8-isoprostone and the inflammatory cytokine IL-6.\(^{70,71}\)

Allopurinol

Allopurinol and its metabolite, oxypurinol, are xanthine oxidoreductase inhibitors that lower serum uric acid levels. Treatment with allopurinol blocks uric acid reabsorption by urate transporters in the proximal tubule, facilitating enhanced uric acid excretion.\(^{72}\) Allopurinol has a protective effect in diseases involving oxidative stress in their pathogen-
Coenzyme Q₁₀ and mitochondrial-targeted anti-oxidants

The kidneys contain the highest endogenous levels of CoQ₉ and CoQ₁₀ compared with all other organs. This is likely due to the reliance of the kidney on aerobic metabolism and high density of mitochondria. It is imperative that endogenous CoQ₁₀ levels are maintained to ensure mitochondrial health, and this forms the rationale for CoQ₁₀ therapy. CoQ₁₀ has three well-characterized functions: (i) the transfer of electrons from complexes I and II to complex III along the ETC of the inner mitochondrial membrane and subsequent membrane polarization and ATP generation; (ii) the pro-oxidant generation of O₂⁻ and H₂O₂; and (iii) the anti-oxidant quenching of free radicals. The major direct anti-oxidant role of CoQ₁₀ is prevention of lipid peroxidation, and it also acts indirectly through its interactions with α-tocopherol. Although results regarding its benefit are disparate, Ishikawa and colleagues demonstrated a decrease in kidney O₂⁻ levels and improved renal function in hemi-nephrectomized rats on a CoQ₁₀ supplemented diet. There is a general lack of human studies investigating CoQ₁₀ therapy for the treatment and/or prevention of CKD, but CoQ₁₀ levels decrease with age and identification of patients with low CoQ₁₀ levels may allow for targeted therapy with beneficial outcome.

Mitochondrial-targeted compounds have created much interest for their application in reducing oxidative stress. One of the most tested is the mitochondrial-targeted derivative of endogenous CoQ₁₀, termed MitoQ (mitoquinone mesylate). This compound and those alike, such as mitochondrial-targeted vitamin E (MitoVitE), are prepared by covalently attaching a lipophilic cation to an alkyltriphenylphosphonium, allowing rapid accumulation into the mitochondria driven by the large negative value of the mitochondrial membrane potential. Administration of MitoQ in a rat model of diabetic nephropathy decreased mesangial expansion and tubulointerstitial fibrosis, thereby improving renal function. Human trials have shown that MitoQ can decrease biomarkers of liver inflammation in hepatitis C patients. However, a larger scale, double-blinded, placebo-controlled study found that MitoQ did not slow the progression of untreated Parkinson’s disease, a disease associated with mitochondrial oxidative stress. The effect may be tissue specific, and early intervention with MitoQ in CKD patients could prove fruitful given that oxidative stress is evident early in CKD progression (stages 1–3).

SUMMARY

The transcriptional networks that maintain oxidant balance in the mature kidney provide promising entry points for future therapeutic interventions, including for CKD. The use of anti-oxidants targeted to specific pathways that are altered in CKD may prove beneficial, but it is likely that several anti-oxidants will be needed as a multi-drug therapy to target oxidant modifying pathways during the development of CKD. These include lipid peroxidation, which can be improved by α-tocopheryl; glutathione redox regulation, which can be restored by NAC; uremic toxins, which can be reduced by allopurinol; inflammation, which can be attenuated by ω-3 polyunsaturated fatty acids; and finally, mitochondrial dysfunction, which may be improved by CoQ₁₀. Mosca and colleagues found that healthy individuals taking a combination of α-tocopherol, α-lipoic acid, CoQ₁₀, carnitines and selenomethionine increased plasma anti-oxidant status, decreased lymphocyte apoptosis and decreased mitochondrial-derived ROS. In the CKD population, identification of patients who would benefit from anti-oxidant therapy is first needed, and then a multifaceted anti-oxidant approach may be necessary for successful treatment of CKD.

REFERENCES

46. Sim AS, Salonikas C, Naidoo D, Wilcken DE. Improved method for plasma malondialdehyde measurement by high-performance liquid chromatography using methyl malondialdehyde as an internal


