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Review article

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Oxidative stress and the role of redox signalling in chronic kidney disease

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Abstract

Chronic kidney disease (CKD) is a major public health concern, underscoring a need to identify pathogenic mechanisms and potential therapeutic targets. Reactive oxygen species (ROS) are derivatives of oxygen molecules that are generated during aerobic metabolism and are involved in a variety of cellular functions that are governed by redox conditions. Low levels of ROS are required for diverse processes, including intracellular signal transduction, metabolism, immune and hypoxic responses, and transcriptional regulation. However, excess ROS can be pathological, and contribute to the development and progression of chronic diseases. Despite evidence linking elevated levels of ROS to CKD development and progression, the use of low-molecular-weight antioxidants to remove ROS has not been successful in preventing or slowing disease progression. More recent advances have enabled evaluation of the molecular interactions between specific ROS and their targets in redox signalling pathways. Such studies may pave the way for the development of sophisticated treatments that allow the selective control of specific ROS-mediated signalling pathways.

Sections

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Key points

- Oxidative stress occurs when the generation of oxidants exceeds the metabolizing or degradative capacity of antioxidants.
- Reactive oxygen species (ROS) are radical or molecular species that contain oxygen and are produced by cellular organelles; they can cause damage to cells and tissues when present in excessive amounts.
- Although potentially harmful in excess, ROS also contribute to cell survival and can act as signalling molecules at appropriate intracellular concentrations.
- Within the kidney, ROS are produced by a variety of organelles and enzyme systems, and contribute to a range of physiological and pathological processes.
- The involvement of ROS in pathological processes suggests that therapeutic targeting of ROS may be beneficial; a number of therapeutic approaches are under investigation, including targeting of Nrf2.

Introduction

Oxidative stress is defined as a state in which the oxidative capacity exceeds the antioxidant capacity of a body or system[1]. It may occur due to the increased production of reactive oxygen species (ROS), a reduction in antioxidant capacity, or a combination of both. ROS are unstable oxygen-containing molecules that readily react with other molecules within a cell. They are generated through processes such as respiration, inflammation and exposure to radiation, UV light and chemicals such as smoke and asbestos, or intense physical activity. In 1956, Harman proposed that ROS contribute to the ageing process[2]. Since then, numerous studies have investigated the effects of ROS. Traditionally, ROS have been viewed as harmful molecules that cause oxidative damage to lipids, proteins and DNA through their reactive properties. However, emerging evidence has revealed that ROS also function as important secondary messengers in cellular signalling pathways[3,4]. For example, cytoplasmic ROS induce the activity of AMP-activated protein kinase (AMPK), which has a crucial role in regulating cellular metabolism[5]. Mitochondrial ROS (mtROS) can also affect HIF1α stability and cell proliferation[6]. Oxidative stress can also activate the transcription factor NF-κB, which induces the expression of cytokines and chemokines to regulate inflammation[7]. Chronic kidney disease (CKD) is characterized by both oxidative stress and inflammation, both of which not only contribute to the progression of CKD but can also lead to the development of cardiovascular disease and other complications[8]. Reports have also demonstrated the involvement of ROS-producing enzymes, oxidants, including hydrogen peroxide (H_2O_2), and dysregulation of the NO synthase (NOS) pathway in pathological conditions, including kidney disease[8–11]. In this Review, we describe the fundamental relevance of ROS to biological processes, including physiological processes and disease pathogenesis with a focus on kidney disease. We also outline the current status of clinical trials that aim to target ROS signals in humans.

The formation and regulation of ROS

Classification of ROS by type and chemistry

The oxygen molecule that is essential for sustaining life is known as triplet oxygen, which exists in its ground state and is represented by the formula $^3O_2$. ROS is a term used to describe a group of reactive molecules that are formed from oxygen molecules. ROS can be divided into non-radical species and free radical species, with the latter defined by the presence of an unpaired electron, making them highly unstable and reactive[11] (Table 1). In the narrowest sense, four types of ROS exist: singlet oxygen ($^1O_2$), which has strong oxidative properties and is produced when $^3O_2$ absorbs energy and becomes excited to the singlet state; superoxide anion $O_2^{\bullet-}$, which is a one-electron reduced form of oxygen; H_2O_2, which is a two-electron reduced form of superoxide; and hydroxyl radicals ($HO^{\bullet}$), which is formed from H_2O_2 (ref. 12) (Fig. 1). The primary and most abundant intracellular ROS is superoxide anion, which is produced by the mitochondrial respiratory chain and through the actions of the NADPH oxidase (NOX) family[13]; its accumulation is commonly associated with oxidative stress. Superoxide can be converted to H_2O_2 by superoxide dismutases (SODs); H_2O_2 itself is also recognized as a major ROS that is involved in redox control of biological activities[14]. In addition, several other oxygen-containing non-radical and free radical species, including organic hydroperoxides (ROOH), alkoxyl radicals ($RO^{\bullet}$) and peroxyl radicals ($ROO^{\bullet}$), can oxidize essential cell components. Moreover, superoxide anion can scavenge nitric oxide (NO) directly or react with NO to form peroxynitrite ($ONOO^-$), a highly reactive nitrogen species (RNS) that can damage proteins, lipids and DNA. Other RNS include haem–NO, dinitrosyl–iron complexes, *S*-nitrosothiols, NO_2, dinitrogen trioxide, nitrosopersulfides and nitroxyl[15]. Together with the above-mentioned ROS, oxygen-containing species and RNS form a large and important group of active redox agents that have critical roles in several intracellular and extracellular processes[16,17].

ROS generation and regulation

The production of ROS in vivo is largely induced by intracellular biological processes, such as oxidative phosphorylation (OXPHOS) and protein disulfide bridge formation, or through triggers such as foreign substances, microbial invasion and cytokines. Intracellular organelles, mitochondria, the endoplasmic reticulum (ER) and peroxisomes are the main sites of ROS production (Fig. 2a). ROS are produced by a variety of enzymes, including xanthine oxidase (XO), cyclooxygenase, lipoxygenase, NOS, haem oxygenase, peroxygenase, haem protein and NOX[18].

Regardless of their mechanism of production, excessive ROS can damage living organisms and have been linked to carcinogenesis, lifestyle-related diseases and other chronic conditions and ageing. Hydroxyl radicals — which are formed from H_2O_2 and superoxide via the Haber–Weiss or Fenton reactions, using metal ions such as iron and copper as catalysts[19] — are highly reactive and oxidize proteins, lipids, carbohydrates, nucleic acids and other biological components.

Due to their short half-life, the effects of hydroxyl radicals are limited and confined to the vicinity in which they are produced. However, oxidative damage is also limited by the actions of ROS scavenging systems, which regulate levels of H_2O_2 and thus hydroxyl radicals (Fig. 1). Two types of ROS scavenging systems exist: antioxidant enzyme and non-enzyme systems. Key antioxidant enzymes include SOD, catalase (CAT), and glutathione peroxidase (GPX). Three types of SOD exist — cytoplasmic (SOD1), mitochondrial (SOD2), and extracellular matrix/plasma membrane outer surface (SOD3) — which catalyse the dismutation of superoxide through the reaction $2O_2^- + 2H^+ \rightarrow H_2O_2 + O_2$. CAT is present in peroxisomes and catalyses the reaction $2H_2O_2 \rightarrow 2H_2O + O_2$. In the presence of glutathione, GPX degrades H_2O_2 to H_2O[11]. Thioredoxin (TRX) and TRX-related protein are disulfide reductases that have roles in regulating the cellular redox state. TRX is induced by various oxidative stresses and

Table 1 | Classification of ROS and their properties

Category	Reactive oxygen species	Properties
Non-radical	Singlet oxygen ($^1O_2$)	Exhibits strong oxidizing capacity involved in a variety of biological processes
	Hydrogen peroxide (H_2O_2)	Low oxidative capacity; involved in a wide range of physiological processes
	Organic hydroperoxides (ROOH)	Organic compounds that contain a peroxide group (–OOH) bonded to a hydrocarbon group. Includes lipid peroxides derived from polyunsaturated acids (PUFAs) and sterols; involved in immune system signalling and cell death via ferroptosis
	Hypochlorous acid (HOCl) and hypobromous acid (HOBr)	Reactive molecules produced by myeloperoxidase from H_2O_2 in vacuoles of neutrophils for pathogen defence
	Electronically excited carbonyl (R–C=O)	A highly reactive species that is electronically excited, typically by absorbing energy such as light
	Ozone (O_3)	An environmental toxin
	Peroxynitrite ($ONOO^-$)	Formed by the reaction of superoxide with nitric oxide
Free radical	Superoxide ($O_2^{\bullet-}$)	Major source of H_2O_2; low reactivity as radicals
	Hydroxyl radicals ($HO^\bullet$)	Short-lived, and highly oxidative radical that reacts with enzyme proteins, cytoskeletal proteins, lipids, carbohydrates and nucleic acids
	Peroxyl radical ($ROO^\bullet$)	Peroxyl radicals are formed by the direct reaction of oxidized alkyl radicals with oxygen and are also formed by the decomposition of alkyl peroxides; they have a strong oxidative potential and can extract hydrogen from molecules with lower standard reduction potentials; this reaction is often observed during the propagation phase of lipid peroxidation
	Alkoxyl radical ($RO^\bullet$)	Intermediate in lipid peroxidation; moderate reactivity
	Nitric oxide or nitrogen monoxide ($NO^\bullet$)	Major source of H_2O_2; low reactivity as radicals
	Nitrogen dioxide ($NO_2^\bullet$)	From $ONOO^\bullet$ decomposition

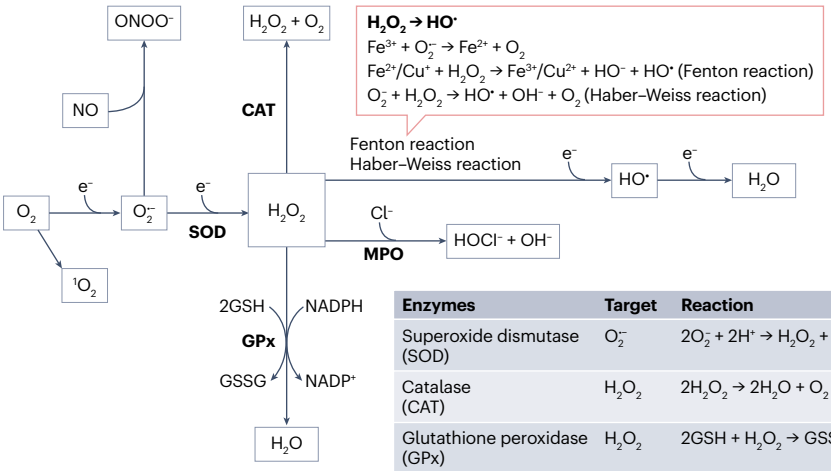
demonstrates ROS scavenging activity in combination with peroxiredoxin (PRX), a TRX-dependent peroxidase. The active site of TRX consists of the sequence –Cys–Gly–Pro–Cys–, and it exists in an oxidized form with an –S=S– bond between two cysteine residues and a reduced form with an –SH–SH. Reduced TRX binds to an oxidized target protein and reduces the disulfide bond of the target protein to a thiol group (–SH). During this process, the thiol group of TRX itself becomes oxidized. The oxidized form of TRX is then reduced by TRX reductase in the presence of NADPH[20]. Several low-molecular-weight compounds with antioxidant properties can also protect from oxidative injury. These compounds can be synthesized by the body or obtained through food. Examples include ascorbic acid, α-tocopherol and bilirubin, which serve to supplement and scavenge radicals[21].

Physiological function and biotoxicity of ROS

Traditionally, oxidative stress has been considered to result from an imbalance between the production and scavenging of ROS, leading to damage to biomolecules, such as DNA, lipids and proteins[22,23]. Although ROS are clearly detrimental to organisms — as evidenced by the finding that mice with mitochondria-specific deletion of *Sod* are unable to survive[24] — a number of studies have also shown that mitochondria-derived and other ROS production pathways are involved in the regulation of a variety of physiological cellular functions. Thus, low concentrations of ROS are produced as needed and can be considered to have a hormesis effect, whereby it is harmful at high concentrations but beneficial at low concentrations[25,26]. Aquaporins are responsible for transporting H_2O_2 across membranes to facilitate redox signalling. However, the spatial distribution of H_2O_2 is not uniform. The concentration of H_2O_2 in plasma is estimated to be approximately 1–5 μM, which is more than 100 times higher than the concentration of H_2O_2 inside cells[11,13]. In addition, concentration gradients exist from the extracellular to the intracellular environment and between different organelles within the intracellular environment. The classification of a given H_2O_2 concentration as beneficial or detrimental may depend on factors such as cell type, level of organ complexity or duration of H_2O_2 exposure[13]. On the other hand, normal cell function might also be disrupted when levels of H_2O_2 are too low, potentially owing to a diminished cellular response to stress and a paradoxical increase in oxidative stress (a process known as reductive stress)[27,28] (Fig. 2b).

ROS have numerous physiological roles at low concentrations. For example, the production of superoxide by neutrophils and macrophages contributes to the phagocytic elimination of foreign substances. Oxidative conditions are also essential for the process of autophagy, as evidenced by the finding that starvation of cells in vitro stimulates the production of ROS and H_2O_2, whereas antioxidant treatment inhibits the starvation-induced formation of autophagosomes and associated protein degradation[29]. The cysteine protease HsAtg4 is also a direct target of oxidation by H_2O_2, and the presence of HsAtg4 is essential for autophagosome formation[23,29]. Furthermore, H_2O_2 has a crucial role in thyroid biosynthesis. Specifically, the addition of iodine to tyrosine residues on thyroglobulin, which leads to the production of thyroid hormones, requires H_2O_2 as an oxidizing agent[30].

A 2005 study found that hypoxic stabilization of hypoxia-inducible factor 1α (HIF1α) and HIF2α requires complex III of the mitochondrial electron transport chain (ETC) and that an increase in ROS leads to the stabilization of the HIFα[31]. This finding indicates that mitochondria may function as oxygen sensors, signalling the stabilization of HIF1α and HIF2α under hypoxic conditions by releasing ROS into the cytoplasm. According to the free radical theory of ageing, ROS produced by complex III during respiration oxidizes cellular components, such as lipids, proteins and nucleic acids, leading to cellular dysfunction and shortening lifespan[1].



Enzymes	Target	Reaction
Superoxide dismutase (SOD)	$O_2^{•-}$	$2O_2^{•-} + 2H^+ \rightarrow H_2O_2 + O_2$
Catalase (CAT)	H_2O_2	$2H_2O_2 \rightarrow 2H_2O + O_2$
Glutathione peroxidase (GPx)	H_2O_2	$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$
Myeloperoxidase (MPO)	H_2O_2	$H_2O_2 + Cl^- \rightarrow HOCl^- + OH$

Fig. 1 | Generation of ROS and its regulation. Main pathways for the generation and metabolism of reactive oxygen species (ROS). Superoxide ($O_2^{•-}$) is converted to H_2O_2 by superoxide dismutase (SOD). Hydrogen peroxide (H_2O_2) is eliminated by catalase (CAT) and glutathione peroxidase (GPx). Myeloperoxidase (MPO) produces hydroxyl radicals ($HO^•$) and hypochlorous acid (HOCl) from H_2O_2. The Fenton and Haber–Weiss reactions are chemical processes that generate ROS in biological systems, particularly within cells. In the Fenton reaction hydrogen peroxide reacts with metal ions to produce hydroxyl radicals, whereas in the Haber–Weiss reaction hydroxyl radicals are produced from H_2O_2 and O_2^-. GSH, glutathione; GSSG, glutathione disulfide.

The lifespan-extending effects of caloric restriction have been demonstrated in studies in non-human primates and human populations with phenotypes common to calorie-restricted monkeys and rodents[32–34]. One study investigated the reduction of oxidative stress in mitochondria in the anti-aging mechanism of calorie restriction both in vivo and in vitro. Calorie restriction reduced oxidative stress and simultaneously stimulated mitochondrial proliferation through phosphorylation of the peroxisome proliferator-activated receptor-γ coactivator 1α (PGC1α) signalling pathway[35]. In addition, mitochondria consumed less oxygen under conditions of calorie restriction than under normal conditions, had a lower membrane potential and were able to maintain ATP production despite lower levels of ROS[35].

This mechanism may also contribute to the antioxidant and lifespan-extending effects of moderate exercise through energy expenditure. In addition, activation of the NAD-dependent histone deacetylase, sirtuin, can induce the deacetylation of transcription factors such as p53, NF-κB, forkhead box O (FOXO) and PGC1α, potentially contributing to the increase in cell lifespan[36]. The effects of calorie restriction and exercise on kidney disease are controversial. However, it is clear that alterations in oxidative stress contribute to their effects; further research is needed to better understand this relationship. Importantly, ROS and its associated oxidative stress can lead to a decrease in the bioactivity of NO — a short-lived diatomic signalling molecule that is important for endothelial and vascular function.

Antioxidants

Antioxidants protect mammalian cells from damage caused by ROS and related species. They work by directly scavenging ROS, inhibiting their formation or repairing damage caused by ROS. Some antioxidants, such as SOD, are produced endogenously, whereas others, such as vitamin C, must be obtained from the diet[21]. Direct antioxidants are short-lived and have redox activity. They are used up during antioxidant activity and must therefore be replenished or regenerated. Examples of direct antioxidants include antioxidant vitamins, glutathione and *N*-acetylcysteine, which can directly react with and scavenge ROS[21].

Antioxidant stress proteins, such as haem oxygenase 1 (HO1), are also important in protecting tissues and reducing inflammation. HO1 is an enzyme that degrades toxic free haem and produces cytoprotective substances[37]. Haem-containing proteins contribute to essential physiological functions, but upon injury they release free haem, causing oxidative stress, inflammation and apoptosis[38]. HO1 has been shown to confer protection in several models of acute kidney injury (AKI)[39–41]. It degrades haem to produce iron ions, carbon monoxide (CO) and biliverdin, which is then converted to bilirubin by biliverdin reductase[39]. Bilirubin has antioxidant effects, whereas CO has signalling and anti-inflammatory effects[42,43].

Major sources of ROS in the kidney

The kidney has one of the highest rates of oxygen consumption of all organs. Although the kidney comprises only 0.5% of total body weight, it is responsible for about 7% of the total oxygen consumption of the body[44]. Moreover, the mitochondrial density of the kidneys is second only to that of the myocardium[45]. Hence, it is important to consider the relationship between the kidney and ROS. Mitochondria and the NOX family are important sources of endogenous ROS in the kidney (Fig. 2).

Mitochondria

Mitochondria contain both an outer and an inner membrane; the five complexes I–V that make up the ETC are located in the inner membrane and use redox reactions to produce ATP. Specifically, NADH and succinate are oxidized in complex I and complex II, respectively, to reduce ubiquinone to ubiquinol. Ubiquinol is then oxidized in complex III to reduce cytochrome *c*. Cytochrome *c* is then oxidized in complex IV and transfers electrons to oxygen molecules, resulting in the production of water[46]. This process results in the establishment of an H^+ gradient across the inner mitochondrial membrane, which drives the synthesis of ATP. Electrons that are leaked during electron transfer reduce oxygen molecules, resulting in the production of superoxide. Superoxide that is generated in the intermembrane space of mitochondria is converted to oxygen and H_2O_2 by SOD1, whereas superoxide that is generated in the mitochondrial matrix is converted by SOD2. H_2O_2 is then reduced to water by GPX and PRX. Thus, ROS are continuously produced by mitochondria, but are eliminated by antioxidant enzymes to maintain redox balance. However, when the excess production of ROS exceeds antioxidant capacity due to ageing or disease, the redox balance is disturbed, leading to oxidative stress[47].

ER and peroxisomes

Within the ER, H_2O_2 is mainly produced by ER oxidoreductin 1 (ERO1) during the formation of disulfide bonds in oxidative protein folding[48]. In addition, NOX4 also produces H_2O_2 in the ER[49]. Similar to mitochondria and the ER, peroxisomes are organelles that contain antioxidant enzymes such as CAT, SOD1 and PRX5, which maintain the balance of oxidative levels in the cell[49]. Fatty acid β-oxidation by acyl-CoA oxidase (ACOX) is also a major source of H_2O_2 in peroxisomes[50]. Several enzymes in peroxisomes, including XO, D-amino acid oxidase, D-aspartate oxidase, L-pipecolic acid oxidase, L-α-hydroxyacid oxidase, and polyamine oxidase, also contribute to the production of peroxisomal ROS. Peroxisomes are abundant in the kidney, particularly in the proximal tubules, with few present in the glomerulus, distal tubules and collecting ducts[51,52].

NADPH oxidases

Seven homologues of NOX exist: NOX1–NOX5, DUOX1 and DUOX2. Of these, NOX2 and NOX4 are highly expressed in the kidney[53]. NOX2 is a six-transmembrane protein that is anchored to the plasma membrane and has cytosolic amino-terminal and carboxy-terminal ends. Activation of NOX2 requires its interaction with p22phox and the translocation of p47phox, p67phox and p40phox to the plasma membrane[54], resulting in Rac-mediated activation of its effector, p67phox, and NOX2 activation. Three Rac GTPases exist: Rac1, which is mainly found in peripheral tissues; Rac2, which is mainly distributed in myeloid cells; and Rac3, which is mainly found in the central nervous system.

In the kidney, NOX2 is involved in tubular functions, such as electrolyte transport and glucose handling[53]. Both NOX2 and NOX4 are also a source of the endothelium-derived vasodilator H_2O_2 in the renal artery[55]. High expression of *Nox2* has been observed in several mouse models of diabetes[56,57]. In C57BL/KsJ diabetic mice, downregulation of *Nox2* expression with candesartan and pioglitazone led to an increase in SOD expression, decreased oxidative stress, improved glucose tolerance and reduced kidney and heart fibrosis[56]. Similarly, treatment of diabetic Akita mice with a human recombinant angiotensin-converting enzyme 2 attenuated levels of NOX2 in the kidney, and decreased renal oxidative stress, urinary albumin, renal fibrosis and systolic blood pressure[57]. These findings suggest that NOX2 may have a role in the development of renal oxidative stress in diabetes and the development of diabetic kidney disease (DKD)[53].

Like NOX2, activation of NOX4 requires it to bind the transmembrane protein p22phox. However, in addition to localizing to the plasma membrane, NOX4 also localizes to intracellular membranes, including mitochondria, the ER, focal adhesions and nuclei[58]. Aberrant upregulation of NOX4 has been proposed to have a pathogenic role in various kidney diseases, including DKD, hypertension-associated kidney disease and polycystic kidney disease, by increasing ROS production and causing mitochondrial damage[58]. However, interstitial fibrosis and oxidative stress were exacerbated following unilateral ureteral obstruction (UUO) in *Nox4*-knockout mice compared with wild-type mice[59], suggesting that abnormally high or low concentrations of ROS may have pathological consequences. Whether NOX4 has a net positive or negative effect in the kidney remains unclear.

Xanthine oxidase

Xanthine oxidoreductase (XOR) is a member of the highly conserved family of molybdoflavoenzymes, which are widely distributed in prokaryotic and eukaryotic organisms. In mammals, XOR is distributed in various organs, including the liver and vascular endothelial cells, and is also abundant in the mammary gland. Its main role is the catabolism of nucleotides, including adenine and guanine[60]. In mammals, XOR is

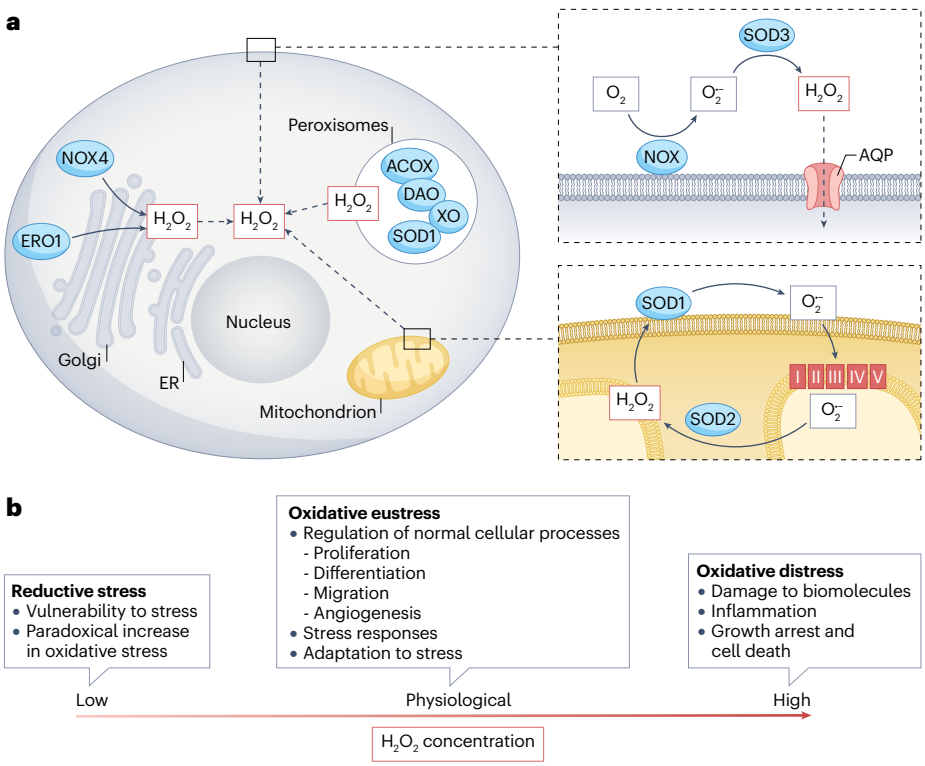


Fig. 2 | Sources of ROS production and their consequences. a, Mitochondria, the endoplasmic reticulum (ER) and peroxisomes are the primary sites of reactive oxygen species (ROS) production. Enzymes and enzyme complexes involved in this process include ER oxidoreductin 1 (ERO1), superoxide dismutase (SOD), acyl-CoA oxidase (ACOX), xanthine oxidase (XO), D-amino acid oxidase (DAO) and NADPH oxidase (NOX). Aquaporins (AQP) can transport H_2O_2 across membranes; however, H_2O_2 can also diffuse through lipid membranes such as cell membranes and the membranes of intracellular organelles. **b**, The effects of ROS on cellular processes differ according to their concentration. Generally, low concentrations of H_2O_2 are necessary for normal life processes and can induce responses to stress, whereas high concentrations can cause inflammatory reactions leading to growth arrest and cell death. However, very low levels can also be detrimental.

post-translationally converted to xanthine dehydrogenase, which accepts NAD^+ as an electron acceptor, or XO[61]. Circulating XO can adhere to endothelial cells by binding to endothelial glycosaminoglycans, and donates electrons to molecular oxygen, creating O_2^- and H_2O_2 (ref. 62).

XO activation may lead to the excessive production of ROS and cellular dysfunction. A clinical trial that compared the effects of XO inhibitors and urate-lowering drugs on endothelial cell function in patients with heart failure demonstrated an improvement in endothelial function only with the XO inhibitor, as a consequence of reduced oxidative stress[63]. In line with this finding, we have shown that XO inhibitors preserve glomerular endothelial function and rescue impaired glomerular permeability in diabetic mice, suggesting that XO activation has an important role in the pathogenesis of DKD[64]. Thus, XO is also a potential therapeutic target for vascular diseases, including those that affect the kidney.

Myeloperoxidase

Myeloperoxidase (MPO) is a haem-containing peroxidase that is synthesized during myeloid differentiation and stored in the azurophil granules of leukocytes. In contrast to O_2^-, which in phagocytes is produced by NOX2 and is released out of the cell, MPO generates hypochlorous acid (HOCl) and other oxidants that are used by the phagocytes to kill pathogens[65]. MPO-derived oxidants are also implicated in the formation of neutrophil extracellular traps, which support the capture and killing of bacteria[66,67]. Typically, MPO catalyses the oxidation of halide ions via H_2O_2 to produce HOCl. However, in various diseases, MPO is released extracellularly by degranulation, and oxidizes not only halide ions but also other substrates to mediate tissue damage[68]. MPO contributes to the oxidative modification of LDL by catalysing lipid peroxidation[69], and available evidence suggests a correlation between circulating blood MPO concentrations and MPO-derived oxidized molecules and coronary artery disease[70]. Microscopic polyangiitis (MPA) is a systemic small-vessel vasculitis that primarily affects the kidneys, lungs and nerves[71]. In Japan and other parts of Asia, many cases of MPA are associated with anti-neutrophil cytoplasmic autoantibodies (ANCAs), with antigen specificity for MPO (MPO–ANCA)[72,73]. MPO–ANCA binds to MPO and recognizes specific epitopes on the surface of polymorphonuclear neutrophils. Subsequent activation of neutrophils increases the binding of polymorphonuclear neutrophils to the endothelium and the generation of superoxide anion radicals and H_2O_2 via oxidative bursts. ANCA-positive necrotizing glomerulonephritis is associated with marked infiltration of the glomerulus by neutrophils and monocytes, which can lead to rapidly progressive glomerulonephritis[74].

NOS

NO and other bioactive nitrogen species have important roles in various physiological functions such as the kidney, cardiovascular and metabolic systems[15]. NO is produced by L-arginine-dependent NOS and can also be created through the nitrate–nitrite–NO pathway, which involves the serial reduction of inorganic nitrate and nitrite[75,76]. The NOS pathway is the primary means by which endogenous NO is generated in the body. However, the nitrate–nitrite–NO pathway is particularly important in conditions in which the activity of the NOS system is reduced or non-functional, such as under conditions of hypoxia, ischaemia or low pH. This pathway can be enhanced by dietary means[15].

Three types of NOS exist. Neuronal NOS (nNOS; also known as NOS1) and endothelial NOS (eNOS; also known as NOS3) are constitutively expressed, whereas inducible NOS (iNOS; also known as NOS2) is associated with inflammatory conditions. The Human Protein Atlas and other findings indicate that nNOS is expressed in cortical tubules, whereas eNOS is expressed in glomeruli[15]. The expression of iNOS in the normal kidney is controversial[77]. Although several studies have demonstrated constitutive expression of iNOS, other studies have found that iNOS protein and mRNA levels increase dramatically in response to stimuli such as ischaemia–reperfusion injury (IRI)[78], sepsis[79] and haemorrhagic shock[80].

Regardless of its mode of generation, NO binds to the reduced haem site of soluble guanylyl cyclase (sGC), activating the enzyme and inducing production of the second messenger cGMP from GTP. NO has numerous beneficial effects on the cardiovascular, renal and metabolic systems, mainly through cGMP-dependent mechanisms, although cGMP-independent mechanisms have also been reported. These mechanisms include modulation of protein function and immune activity, reduction of angiotensin II (ANGII) signalling, modulation of oxidative stress and sympathetic nerve activity, and regulation of mitochondrial function. In the pathogenesis of cardiovascular disease and CKD, reduced NO bioactivity is associated with organ dysfunction. Reduced NO bioactivity can be attributed to several factors, including reduced NOS expression, limited substrate availability, uncoupling of NOS (a phenomenon in which the normal function of NOS is disrupted, resulting in the production of ROS such as superoxide and H_2O_2 instead of NO), increased levels of endogenous NOS inhibitors such as ADMA, and impaired signalling under conditions of oxidative stress[15,81]. In the kidney, NO is critically involved in the autoregulation and modulation of tubular transport, which may be important in the development and progression of hypertension, CKD, IRI and cardiovascular disease[15].

Endothelial NOS. Endothelium-derived NO, produced by eNOS, has a critical role in regulating blood flow and maintaining endothelial integrity. Mice that do not express *eNos* show symptoms similar to those of the metabolic syndrome and develop severe kidney disease[76,82,83]. Of interest, the metabolic syndrome of *eNos*-deficient mice can be reversed by dietary nitrate supplementation, which activates the nitrate–nitrite–NO pathway[84]. The uncoupling of eNOS and activation of NOX are major contributors to the production of ROS[10], and have been linked to the initiation and progression of DKD[85,86]. Furthermore, our studies suggest a possible link between impaired endothelium-derived NO activity, the production of ROS resulting from eNOS uncoupling, and endothelial dysfunction in models of DKD[10,87,88].

Neuronal NOS. NOS1 (nNOS) and NOX2 are the main regulators of tubuloglomerular feedback (TGF) — a tightly coordinated mechanism by which the kidney balances sodium excretion. Scavenging by NOX2-derived ROS attenuates the bioavailability and signalling of NOS1-derived NO[89]. In situations characterized by oxidative stress, TGF activity is increased. Conversely, conditions characterized by increased NO production can inhibit the TGF response[89–91].

The β-splice variant of NOS1 that is expressed in macula densa cells (NOS1β) is particularly important in NO production[92]. The expression and activity of NOS1β is altered by factors such as high salt intake, hypertension and diabetes[89,92–94]. NOS1β-mediated regulation of TGF and renal haemodynamic responses has also been shown to have an important role in pregnancy[95].

The role of ROS in inflammation

Uncoupling of NOS and ROS–NO imbalance

Uncoupling of eNOS results in the generation of ROS rather than NO, contributing to vascular endothelial dysfunction[96,97] (Fig. 3).

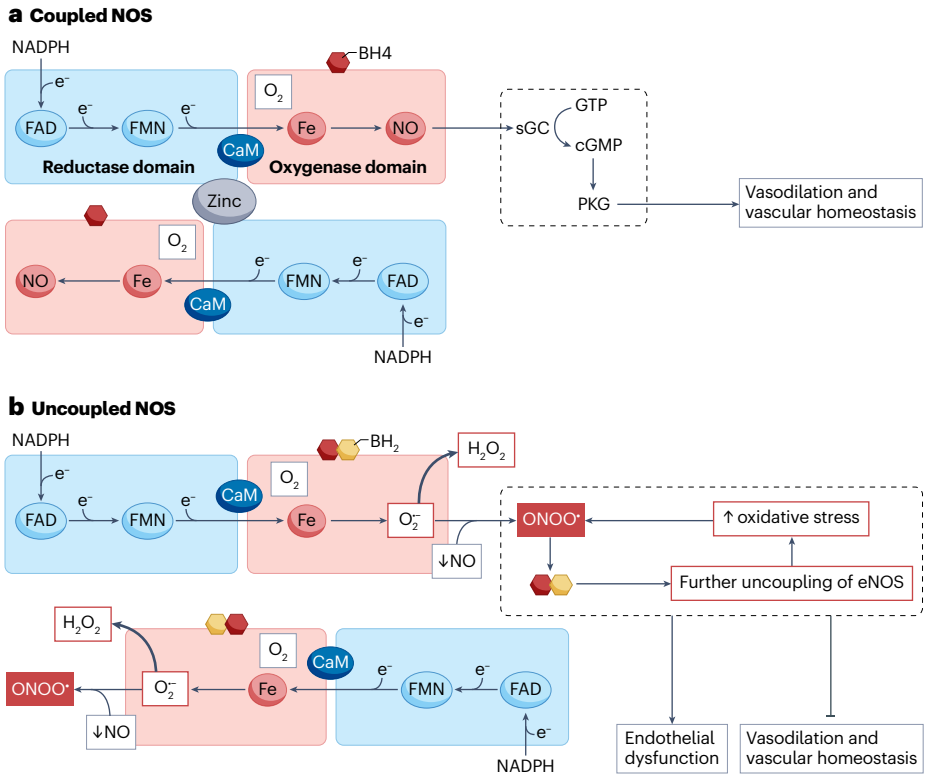


Fig. 3 | Uncoupling of eNOS. a, Endothelial nitric oxide (NO) synthase (eNOS) is considered to be coupled when it exists as a dimer, which produces abundant levels of NO and a small amount of reactive oxygen species (ROS). Tetrahydrobiopterin (BH_4) binds to the oxygenase domain of eNOS to stabilize the dimer, whereas calmodulin (CaM) regulates the flow of electrons from NADPH in the reductase domain to the haem in the oxygenase domain. Zinc ions bound to eNOS are also required for dimer formation and stability. NO diffuses into vascular smooth muscle cells (VSMCs) where it binds to soluble guanylyl cyclase (sGC) and initiates the synthesis of 3,5-cGMP. This in turn activates cGMP-dependent protein kinase G (PKG), which promotes VSMC relaxation, resulting in vasodilation. **b**, The absence of BH_4, the oxidation of BH_4 to dihydrobiopterin (BH_2), or low levels of heat shock protein 90 can induce uncoupling of the eNOS dimer to form two monomers, which are less efficient at producing NO and generate large amounts of ROS. In addition, peroxynitrite, produced by the reaction of superoxide with NO, oxidizes BH_4. This process leads to a reduction in the cellular ratio of BH_4 to BH_2, exacerbating eNOS uncoupling and perpetuating a deleterious cycle of oxidative stress. FAD, flavin adenine dinucleotide; FMN, flavin mononucleotide.

Several factors can trigger eNOS uncoupling, including: depletion of the critical eNOS cofactor tetrahydrobiopterin (BH_4) as a consequence of oxidative stress; deficiency of the eNOS substrate L-arginine; accumulation of its analogue asymmetric dimethylarginine (ADMA); and eNOS S-glutathionylation[98].

NOS has both oxidizing and reducing properties and requires BH_4 to function as a NOS. In the context of low L-arginine or BH_4 levels, NOS cannot dimerize and becomes unstable, which results in the donation of electrons to oxygen instead of L-arginine, and leads to the formation of ROS[5]. Dihydrobiopterin (BH_2) is the oxidized form of BH_4; it is produced in response to cardiovascular disease-associated oxidative stress[99,100], resulting in a decrease in the ratio of BH_4 to BH_2. A reduction in BH_4 levels, along with a decrease in the BH_4 to BH_2 ratio, is believed to be a major cause of eNOS uncoupling, and has been linked to endothelial dysfunction in numerous disease models and diseases[98], including coronary artery disease[101], peripheral artery disease[102], diabetes[103], hypertension[104], hypercholesterolaemia[105] and heart failure with preserved ejection fraction[106].

Evidence suggests that BH_4 treatment can prevent eNOS uncoupling in animal models[107–109] and improves endothelial function in humans[104,105,110,111]; however, the consequences of this effect are unclear. All of the above-mentioned human studies were conducted with intravenously administered BH_4. Studies that used orally administered BH_4 found improved endothelial dysfunction in patients with dyslipidaemia[112], hypertension[113] and rheumatoid arthritis[114], but no improvement in patients with coronary artery disease in whom it instead increased BH_2 levels[115]. These different outcomes may reflect the fact that BH_4 is unstable and easily oxidized. Thus, coadministration of BH_4 with antioxidants may be required to restore the BH_4 to BH_2 ratio in patients with high levels of oxidative stress[116]. Furthermore, a study of vascular dysfunction in a model of subarachnoid haemorrhage found that BH_4 levels are dependent of the rate of BH_2 reduction by dihydrofolate reductase, and correlate negatively with levels of NOX4 (ref. 117).

We use an in situ visualization method in which administration of diaminorhodamine and dichlorofluorescein diacetate enables the detection of NO and ROS, respectively, in perfused kidneys by confocal laser microscopy. Arginine and calcium are also administered to activate eNOS. This technique allows the simultaneous analysis of changes in NO and ROS in disease models[10]. In rats with streptozotocin (STZ)-induced diabetes, for example, we found that O_2^- is produced not only by NOX but also by NOS as a consequence of eNOS uncoupling. Administration of BH_4 improved the imbalance between ROS and NOS, and decreased urinary albumin excretion[10,87]. Levels of the enzyme that is responsible for BH_4 synthesis — guanosine triphosphate cyclohydrolase I (GTPCH I) — are often reduced in diabetes. We have shown that maintaining levels of GTPCH I in the glomerular endothelium can ameliorate kidney disease in diabetic Akita mice[88].

L-Arginine supplementation is another potential approach to preventing eNOS uncoupling and increasing NO synthesis in the endothelium. It was once considered a promising therapeutic approach to preventing cardiovascular disease[98,118,119]; however, the results of experimental and clinical studies have been mixed[98]. Lastly, S-glutathionylation of eNOS in endothelial cells is also linked to defects in vasodilation as a consequence of reduced NO production and increased ROS generation[120]. This modification can be reversed by thiol-specific reducing agents to restore protein function[121]. Deglutathionylation can be achieved by glutaredoxin 1 and TRX, which have

been shown to improve endothelial function and prevent myocardial infarction[122,123]; however, further research is needed in this area[98].

Keap1–Nrf2 system

Various mechanisms are used to produce the enzymes required to respond to changes in the environment. The HIF pathway and the Keap1–Nrf2 system are two such mechanisms — both of which may represent therapeutic targets in the treatment of kidney disease[124,125] (Fig. 4a). NRF2 is a basic leucine zipper-type transcription factor that maintains homeostasis in the body mainly in response to oxidative stress. In its inactive state, NRF2 localizes to the cytoplasm and is bound to Kelch-like erythroid cell-derived protein with CNC homology-associated protein 1 (KEAP1). KEAP1 is a highly sensitive sensor of electrophilic materials, which induce the ubiquitination and subsequent degradation of NRF2 by a cullin-type E3 ubiquitin ligase. This degradation process maintains constant, low concentrations of NRF2. However, oxidative stress modifies a cystine residue in Keap1, which alters its structure and enables NRF2 to escape degradation. NRF2 subsequently enters the nucleus[126], where it binds to small musculo-aponeurotic fibrosarcoma oncogene homologue and antioxidant responsive elements in the promoter region of antioxidant genes to induce the expression of proteins such as glutathione synthase and HO1, which are essential for the protection of cells and organs from oxidative stress[124].

Studies in animal models with genetic modification of Keap1–Nrf2 components have consistently shown that NRF2 attenuates oxidative stress caused by pathological conditions, including IRI, UUO, nephrotoxins and diabetes, and slows the progression of associated kidney diseases[127–129]. This research supports the notion that oxidative stress is a key factor in both the onset and progression of kidney disease and highlights the importance of oxidative stress accumulation as a common pathway in various models of kidney disease[127–129]. However, decreased NRF2 activity and a reduction in its target gene products, including antioxidant enzymes, the key enzymes responsible for glutathione synthesis, and the major detoxifying enzyme NQO1, have been observed in models of CKD, including rats with five-sixths nephrectomy-induced CKD, despite the presence of oxidative stress and inflammation in the kidney[8,130]. Reduced Nrf2 activity was also observed in the kidneys of rats that spontaneously developed focal glomerulosclerosis, leading to kidney failure[131]. The observed decrease in Nrf2 activity across various CKD models suggests that inactivation of Nrf2 mediates the onset and progression of CKD across a variety of disease aetiologies[8].

FOXO proteins

The FOXO family of transcription factors maintains cellular and organismal homeostasis in response to various stressors including oxidants[124] (Fig. 4b). The transcriptional activity of FOXO is classically regulated by its serine/threonine kinase AKT-mediated phosphorylation, which induces its translocation from the nucleus to the cytoplasm[132]; however, FOXO can also be subject to direct redox regulation. The function of FOXO is further regulated by a variety of factors. FOXO can dimerize

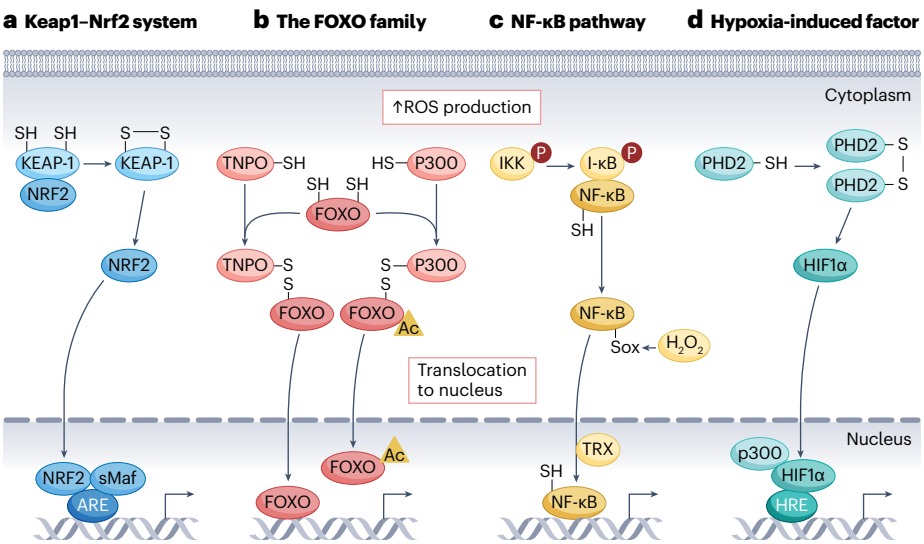


Fig. 4 | Role of ROS in cell signalling. a, Reactive oxygen species (ROS) can induce the formation of intramolecular disulfide bonds in Kelch-like ECH-associated protein 1 (KEAP-1), which enables NF-E2-related factor 2 (NRF2) to escape proteolytic degradation and translocate into the nucleus, where it forms a heterodimer with small Maf protein (sMaf) factor, which binds to the antioxidant response element (ARE) to activate the transcription of target genes involved in the antioxidant response. **b**, Under oxidative stress, forkhead box O (FOXO) forms intermolecular disulfide bonds with the TNPO transporter to facilitate its nuclear transfer and the transcription of genes involved in the antioxidant response. Alternatively, FOXO interacts with the acetyltransferase p300 to acetylate it. This reduces the DNA-binding capacity of FOXO. **c**, Under physiological conditions, nuclear factor-κB (NF-κB) is kept inactive in the cytoplasm by binding to inhibitor of κB (I-κB). During oxidative stress, H_2O_2 activates I-κB kinase (IKK), which leads to the phosphorylation and degradation of I-κB, and the release of NF-κB. NF-κB can then translocate to the nucleus where it activates the transcription of genes involved in inflammatory pathways. In addition, H_2O_2 can also oxidize NF-κB — a modification that can be reversed by the thioredoxin (TRX) in the nucleus to restore DNA binding capacity. **d**, Prolyl hydroxylase domain-containing protein 2 (PHD2) acts as a redox sensor and can hydroxylate and inactivate the transcription factor, hypoxia-inducible factor 1α (HIF1α). Increased ROS production leads to the formation of intramolecular disulfide bonds and the formation of a PHD2 homodimer, resulting in HIF1α stabilization and translocation into the nucleus where it interacts with cofactors such as p300 and the Pol II complex to activate the hypoxic response element (HRE) of hypoxia-responsive genes.

with the TNPO transporter to facilitate its nuclear translocation and enhance FOXO activity[124,133]. Additionally, ROS have been shown to induce the formation of a cysteine–thiol–disulfide-dependent complex between FOXO and p300/CBP acetyltransferase. Acetylation of FOXO by the acetyltransferase p300 reduces its DNA binding and transcriptional capacity. This process is regulated by the TRX system of disulfide reductases[134].

FOXO1 is highly expressed in insulin-responsive tissues such as the liver, adipose tissue, skeletal muscle and pancreas, and is thought to be a key regulator of insulin signalling and glucose homeostasis because it coordinates transcriptional cascades that regulate glucose metabolism. Some studies have suggested that genetic variations in *FOXO1* may increase the risk of DKD[135].

NF-κB pathway

NF-κB is a widely expressed transcription factor that controls the signal-induced expression of genes involved in a variety of biological processes, such as the immune response, inflammation, cell growth and survival[124] (Fig. 4c). Under unstimulated conditions, NF-κB localizes to the cytoplasm where it is bound to inhibitory proteins (members of the I-κB family). Activation of the I-κB kinase complex by ligands that activate Toll-like receptors (TLRs), such as TNF, IL-1β and lipopolysaccharide, induces the phosphorylation of inhibitory proteins, the degradation of I-κBα and the release of NF-κB, which translocates to the nucleus. In the nucleus, activated NF-κB regulates the expression of genes that encode inflammatory mediators, highlighting the interplay between redox sensitivity and inflammation[136]. Sustained activation of NF-κB has been linked to chronic inflammation in CKD[137,138]. In addition, dysfunctional Nrf2 activity may contribute to the development and exacerbation of renal inflammation in CKD by facilitating the accumulation of hydroperoxides and lipoperoxides in kidney tissue. These factors are powerful activators of NF-κB, establishing a connection between the two signalling pathways[8,139].

The HIF pathway

HIF is a member of the basic helix–loop–helix per–ARNT–sim homology (PAS) family of transcription factors. HIFs consist of an α-chain that undergoes oxygen-dependent degradation and a β-chain that is constantly expressed[124] (Fig. 4d). HIF1 is the most abundant HIF isoform and regulates the global response to hypoxia, whereas HIF2 is more localized and involved in the transcription of specific genes such as erythropoietin (*EPO*). In the hypoxic kidney, HIF1α is expressed in tubular epithelial cells, and HIF2α is expressed mainly in endothelial and interstitial cells[140]. HIF activation has a protective effect in models of AKI, with findings from several studies indicating that small-molecule inhibitors of prolyl hydroxylase (PHD) that stabilize HIF can reduce tubulointerstitial injury[141,142]. In a rat model of renal transplantation, pre-administration of a PHD inhibitor to the donor rat prevented graft injury and improved graft survival[141–143]. HIF activation after ischaemic kidney injury is associated with a reduction in tubular cell apoptosis, inflammatory cell infiltration and peritubular capillary loss[143,144]. Moreover, systemic deletion of *Vhl*, which leads to activation of HIF1 and HIF2, also confers renal protection in AKI models[145]. Conversely, mice with heterozygous knockdown of *Hif1a* and *Hif2a* are more susceptible to IRI than wild-type littermate controls[146]. Of interest, systemic knockdown of *Hif2a* alone exacerbates tubulointerstitial injury whereas restoration of *Hif2a* in endothelial cells ameliorated IRI-induced kidney damage[147]. The protective effects of PHD inhibitors have been linked to HIF-induced upregulation in glycogen synthesis, which contributes to cell survival under conditions of oxygen and glucose deprivation[148]. However, it is important to note that in studies to date, PHD inhibitors administered prophylactically improved tubulointerstitial injury, but administration after the onset of IRI did not[149,150], suggesting that the beneficial effects of HIF activation in the context of AKI therapy may be time-dependent[144,150].

The effects of HIF have also been studied in models of diabetes. In a model of STZ-induced type I diabetes, HIF1 expression was enhanced by antioxidant treatment, suggesting that oxidative stress may attenuate HIF expression[151]. This hypothesis is supported by a study which found that the induction of the HIF target gene, *VEGF*, in response to hypoxia was attenuated in adipose-derived stem cells obtained from patients on dialysis but not in cells obtained from patients without CKD[152].

In 2019, Japan was the first country to approve the use of a PHD inhibitor for the treatment of renal anaemia in patients on dialysis. PHD inhibitors have since been approved for use in patients with non-dialysis-dependent CKD. Together, these findings suggest that dysregulation of the HIF response in CKD and enhanced HIF-mediated signalling may modulate the pathogenesis of CKD.

ROS and intracellular organelle function

ER stress and peroxisomes

The ER serves as a site for the folding and post-translational modification of most membrane and secretory proteins. Indeed, approximately 30% of all newly synthesized proteins undergo some form of modification within the ER, leading to their maturation. ER stress can occur when proteins fail to fold properly within the ER lumen, often as a consequence of changes in the cellular environment, which results in the accumulation of defective proteins. ER stress is associated with an increase in protein disulfide bonds, which stimulates ROS production within the ER[153], but can also promote ROS production within mitochondria by disrupting Ca^{2+} homeostasis between the ER and cytosol. Thus, oxidative stress and ER stress contribute to a detrimental cycle[154]. The accumulation of denatured or misfolded proteins in the ER is detected by sensor proteins such as PERK, IRE1 and ATF6, and activates a response called the unfolded protein response (UPR). Several studies have demonstrated a relationship between activation of the UPR and kidney disease[155]. One study identified a small-molecule compound that targets the UPR as a potential treatment for mucin 1 kidney disease, a rare toxic proteinopathy[156], suggesting that similar approaches could have therapeutic implications for other kidney diseases.

Peroxisomes have a role in detoxifying peroxisomal and intracellular ROS through the activity of enzymes such as SOD, GPX and CAT. One study revealed that mitosis-specific phosphorylation of Ser232 on the peroxisomal membrane protein, PEX14, during cell cycling acts as a defence against ROS by increasing cytoplasmic levels of the antioxidant enzyme, CAT[157]. This finding suggests that the phosphorylation of PEX14 helps maintain cellular homeostasis in cooperation with other organelles by, for example, protecting DNA from ROS through the production of CAT during mitosis — a phase in which the nuclear membrane is lost[157]. It has also been reported that oxidative stress induces the phosphorylation of PEX14 and selectively inhibits CAT transport. In other words, the phosphorylation of PEX14 is important for cellular resistance to oxidative stress[158].

Like mitochondria, peroxisomes are vulnerable to functional and structural damage during IRI[159]. Approximately 90% of the CAT activity in cells is concentrated in peroxisomes. The reduction in CAT activity during renal ischaemia is primarily due to enzyme inactivation.

Ischaemia-induced H_2O_2 generation and intracellular acidosis promote the formation of CAT into enzymatically inactive complexes[159,160]. Prolonged ischaemia followed by reperfusion causes structural changes in peroxisomes, ultimately leading to the release of CAT into the cytoplasm and overall organelle deterioration. The suppression of CAT activity during reperfusion is due to decreased proteolysis and synthesis of the CAT protein[159].

Mitochondria

Physiological role of mitochondrial ROS. mtROS were once thought to be cytotoxic and have no physiological function; however, we now know that mtROS are essential for various cellular processes[22]. For example, mtROS may be important for HIF stabilization under hypoxic conditions[23]. Hypoxia alters the microtubule-dependent transport of mitochondria, leading to an accumulation of mitochondria in the perinuclear region[161]. The resulting increase in nuclear ROS enhances the expression of hypoxia-sensitive genes, such as *VEGF*, by oxidizing the hypoxia response elements within their promoters[162]. Beyond their role in the hypoxic response, mtROS also have physiological roles in differentiation, autophagy and immune cell activation[22].

ROS production and mitochondrial dysfunction. mtDNA is more prone to damage from ROS than nuclear DNA. Several reasons probably underlie the higher susceptibility of mtDNA to oxidative damage, including the fact that mitochondria generate ROS, the absence of protective proteins such as histones, and the absence of effective mtDNA repair mechanisms[163,164]. Similar to nuclear DNA, mtDNA can undergo various types of damage, including single-strand and double-strand breaks, the formation of DNA–protein crosslinks, interstrand crosslinks and intrastrand crosslinks. Such damage can impair mitochondrial function, including the ability to synthesize ATP and carry out metabolic processes such as fatty acid oxidation, the tricarboxylic acid cycle, the urea cycle, amino acid metabolism and haem synthesis[165]. Furthermore, oxidative damage caused by ROS can increase the likelihood that mitochondria will release cytochrome *c* and thereby activate the apoptotic cascade[166].

Mitochondrial damage is a common feature of kidney diseases. For example, ROS production is increased immediately following renal IRI, which causes direct oxidative damage to mitochondrial proteins and lipids, leading to impaired ETC function and increased mitochondrial membrane permeability, which further induces mitochondria disruption[167]. mtROS also activate inflammatory signals such as those involving TLRs and the NLRP3 inflammasome, which exacerbate kidney injury[168].

Regulation of cellular energy metabolism is key to mitigating the cellular damage caused by ROS. For example, shifting ATP production from OXPHOS in mitochondria to glycolysis may reduce IRI-induced organ damage[169]. Moreover, we have reported that the antihistamine, meclizine, can reduce IRI and hypoxia-induced ROS by inhibiting the Kennedy pathway of phospholipid biosynthesis[170]. The rapid removal of damaged or dysfunctional mitochondria is also crucial to maintain cellular homeostasis and viability. Mitophagy, a selective autophagic process that removes mitochondria, is activated in renal proximal tubular cells following IRI, and is critical for maintaining mitochondrial quality control, promoting tubular cell survival and preserving renal function during AKI[171].

The overproduction of ROS as a consequence of mitochondrial dysfunction has also been linked to DKD and CKD. Mice with a point mutation in mtDNA that causes ROS overproduction develop diabetes and lymphoma[172], and increased mtROS production and mitochondrial dysfunction has been reported in patients with CKD, particularly in those with DKD[86]. Mechanistically, glucose-induced ROS production contributes to podocyte apoptosis and depletion[173,174]. However, it is important to note that the dogma that high glucose levels lead to increased mitochondrial superoxide production has been challenged by a study in mice with STZ-induced diabetes, which demonstrated that the production of mtROS in the diabetic environment is counter-intuitively reduced[175]. Similar to excess mtROS, which can harm cells in various ways, insufficient levels of ROS can also disrupt signalling pathways and impair the function of redox-dependent proteins. Therefore, normal cellular function relies on an appropriate balance of the production and scavenging of ROS.

Mitochondrial ROS and cell death. Nephron loss in CKD is caused not only by damage to the glomerulus, particularly podocytes but also by tubular cell death, which can induce morphological changes and irreversible kidney dysfunction. Cell death is typically classified as either apoptotic or necrotic. Apoptosis is a non-immunogenic process that eliminates unnecessary cells. Necrosis was once considered to be an unregulated form of cell death that was caused by oxidative or other forms of chemical stress; however, diverse, regulated forms of necrosis are now known to exist[176].

Ferroptosis is one such form of regulated necrosis that is caused by the accumulation of divalent iron (Fe^{2+})-dependent ROS and the enhanced production of lipid peroxides, which promotes the accumulation of oxidized polyunsaturated fatty acids in membrane phospholipids[177]. Ferroptosis contributes to tubular cell death during AKI, as demonstrated by increased levels of tubular cell death in mice lacking the ferroptosis inhibitor, *Gpx4*, and attenuated tubular damage following administration of the inhibitor of ferroptosis, ferrostatin 1 (refs. 178,179). IL-4-induced 1 (IL-4i1), an amino acid oxidase that is secreted by immune cells, and the enzyme FSP1, which converts vitamin K from its oxidized form to its reduced form, also prevent ferroptosis by preventing the oxidation of intracellular lipids[180,181]. Several drugs that are already in clinical use may inhibit ferroptosis by acting as peroxyl radical scavengers, and have demonstrated protective effects in models of AKI[182].

Necroptosis is another form of regulated necrosis that can induce inflammation in the surrounding tissue secondary to rupture of the cell membrane. The necroptotic pathway is initiated by the cytosolic necrosome complex, which comprises receptor-interacting protein kinase 1 (RIP1), RIP3 and mixed lineage kinase domain-like (MLKL) protein. Activation of this complex induces oligomerization of MLKL and its relocation to the cell membrane, where it forms pores[183]. mtROS have been identified as drivers of a shift from apoptosis to necroptosis under conditions of hyperglycaemia[184]. Necroptosis is also an important form of cell death in kidney disease. *Mlkl* is the most prominently expressed tubular epithelial cell gene in models of AKI, and mice with deletion of *Ripk3* and/or *Mlkl* are protected against IRI-induced kidney injury[185]. RIPK3 and MLKL expression in renal biopsy tissue may be useful for AKI risk stratification[185].

mtDNA and the innate immune system. mtDNA has immunostimulatory properties and can directly activate pattern-recognition receptors (PRRs) in the innate immune system to elicit an inflammatory response[186]. For example, the cytosolic release of mtDNA, induced by mtROS and NLRP3 activation, can stimulate the secretion of IL-1β and IL-18 from macrophages[187,188]. The PRR, AIM2, is also involved in the activation of

caspase 1 downstream of mtDNA[187]. Inflammasome activation in conjunction with mtDNA release has been implicated in several pathologies, including atherosclerosis, age-related macular degeneration, mevalonic acid kinase deficiency and certain bacterial infections[189]. Lastly, the cGAS–STING pathway is a key mechanism for detecting cytosolic DNA and activating the innate immune response. DNA from cells exposed to oxidative stress triggers STING activation and enhances immune recognition. Released mtDNA also activates cGAS–STING signalling, and its inhibition can ameliorate models of kidney disease[190,191].

Organelle crosstalk

Although organelles, such as mitochondria and the ER, individually contribute to the production of ROS, it is important to consider that crosstalk exists between them. The interface between mitochondria and ER is called the mitochondria-associated ER membrane (MAM) and contributes to a variety of cell functions such as lipid metabolism, calcium signalling, ER stress, mitochondrial function, apoptosis and autophagy[192]. Abnormalities in MAM structure or function have been implicated in the development and pathogenesis of several diseases, including DKD. For example, kidneys of patients with DKD have reduced expression of the antioxidant, DsbA-L (disulfide-bond-A oxidoreductase-like protein), and demonstrate impaired MAM maintenance[193]. Overexpression of DsbA-L restores MAM integrity and attenuates glucose-induced tubular damage[193]. In addition, mice that lack phosphofurin acidic cluster sorting protein 2 — a protein that normally localizes to the area of contact between the mitochondria and the ER — rapidly develop proteinuria under diabetic conditions, associated with the destruction of MAM regions, ER stress, mitochondrial dysfunction, apoptosis and worsening fibrosis[194].

Chronic kidney diseases

ROS can contribute to kidney disease through a variety of mechanisms. They can directly damage kidney cells, leading to impaired kidney function, inflammation and fibrosis. They can also activate signalling pathways and transcription factors that exacerbate these processes (Fig. 5). Diabetes, hypertension and the metabolic syndrome are all associated with renal oxidative stress and the uncoupling of NOS, resulting in an imbalance between ROS and NO[10,12,87]. This imbalance can lead to glomerular hypertension, endothelial and epithelial cell damage and albuminuria.

The tubulointerstitium is also vulnerable to injury; AKI which can result from tubulointerstitial damage increases the risk of subsequent CKD[176,195]. In the tubulointerstitial region, injury to endothelial cells of the peritubular capillaries can also cause damage to tubular epithelial cells, at least in part, through imbalances in the ratio of ROS to NO, which activates inflammatory and fibrotic signalling pathways such as those that involve NF-κB, inflammasomes and Wnt–β-catenin[196,197] (Fig. 5b).

Targeting ROS pathways

Findings from animal studies suggest that targeting ROS could represent a promising approach for the treatment of cardiovascular and kidney disease[24,198–201]. However, it is important to note that although antioxidants can neutralize free radicals, they do not affect H_2O_2, which is a key player in many pathophysiological processes[5]. Moreover, and as outlined in this Review, ROS have essential roles in maintaining physiological functions, and thus complete elimination of ROS may disrupt cellular homeostasis and exacerbate disease progression[5]. Despite the potential risks, some drugs have demonstrated promising results in reducing oxidative stress in clinical settings (Table 2).

Renin–angiotensin–aldosterone system blockers

The renin–angiotensin–aldosterone system (RAAS) has a key role in the regulation of blood pressure and cardiovascular function. In addition to elevating blood pressure, ANGII, a major component of the RAAS, induces inflammatory responses by stimulating the formation of ROS through the activation of NAD(P)H oxidase. ROS subsequently promote cell growth, the expression of pro-inflammatory genes, and the production of extracellular matrix proteins[202]. Clinical trials have shown that RAAS blockers reduce urinary protein levels and demonstrated renoprotective effects through blood pressure-dependent and blood pressure-independent mechanisms[203–205], including through the attenuation of pro-inflammatory and profibrotic pathways[206]. Studies in genetic models of kidney disease support the notion that the tissue benefits of RAAS blockade go beyond blood pressure control alone, and include improvements in proteinuria, attenuation of podocyte effacement, and reductions in oxidative stress and perivascular fibrosis.

SGLT2 inhibitors

Sodium–glucose cotransporter 2 (SGLT2) inhibitors have gained widespread recognition for their renoprotective effects beyond glucose control. The mechanisms underlying these effects are not clear, but a number of possibilities exist. For example, the inhibition of glucose uptake by proximal tubular cells may reduce oxidative stress[207]. This reduction in oxidative stress may be in part mediated by a parallel induction of uricosuria, through either direct or indirect effects of SGLT2 inhibitors on URAT1 (refs. 208,209). Alternatively, SGLT2 inhibitors may target the molecular pathways responsible for changes in Ca^{2+} signalling and oxidative stress associated with inflammation[210,211]. SGLT2 inhibitors also reportedly improve endothelial function and aortic stiffness[212]. Moreover, SGLT2 inhibitors may directly vasodilate blood vessels and increase the production of NO[212], which could contribute to organ protection by improving endothelial function[209,213].

The NO–sGC–cGMP pathway

As described earlier, total NO production is decreased in kidney disease due to impaired endothelial function and an increase in the endogenous eNOS inhibitor, ADMA. A randomized controlled trial in patients undergoing multiple valve replacement for rheumatic heart disease with no additional risk factors for endothelial dysfunction demonstrated that the administration of NO led to a decrease in stage 3 CKD up to 1 year following surgery[214]. Whether the kidney-protective effects of NO gas are similar or superior in patients with endothelial dysfunction is unclear. A phase III clinical trial (NCT02836899) will investigate the effectiveness of inhaled NO as a treatment for AKI in patients undergoing cardiac surgery[215–217]. The efficacy of NO is limited in patients with heart failure due to excessive oxidative stress and inflammation-induced damage to the vascular endothelium. The sGC stimulator, vericiguat, increases the production of cGMP, which may alleviate heart failure symptoms. In the VICTORIA trial in 5,050 patients with heart failure with reduced ejection fraction, vericiguat treatment resulted in a 10% reduction in the primary end point of cardiovascular death or first hospitalization for heart failure[198]. Such agents are expected to have similar protective effects in the kidney, by increasing renal blood flow and improving GFR, but also through direct attenuation of CKD progression[10,87,88]. Indeed, sGC activators have demonstrated beneficial effects on kidney function in preclinical models of kidney disease, which importantly are not solely dependent on blood pressure reduction[15,218]. Mechanistic studies suggest that the

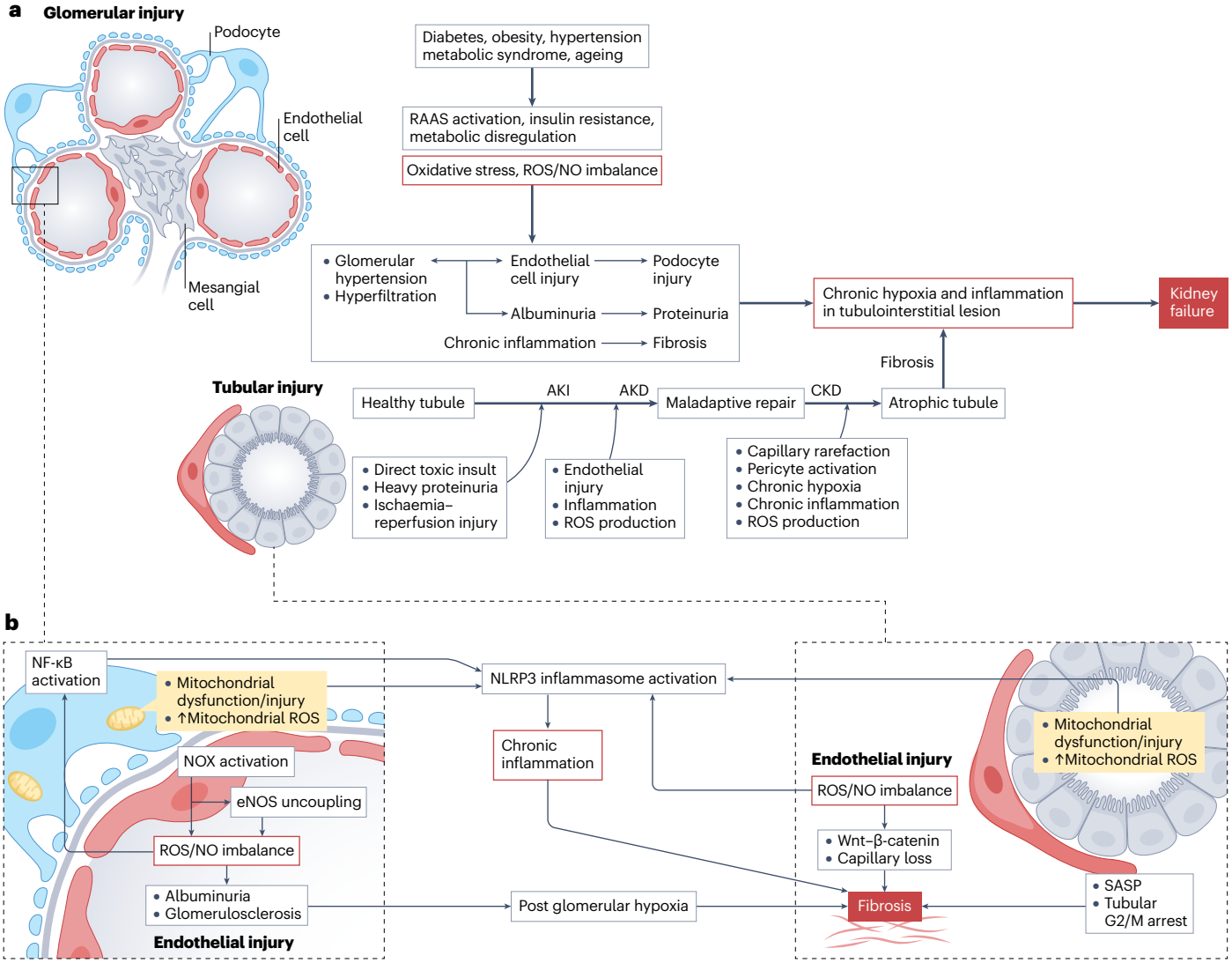


Fig. 5 | Oxidative stress in the pathogenesis and progression of CKD. **a**, Diabetes mellitus, hypertension, the metabolic syndrome and ageing are associated with an imbalance in reactive oxygen species (ROS) and NO, which can contribute to glomerular hypertension, endothelial damage and the development of albuminuria. Progression of glomerular damage leads to a decrease in oxygen supply to the tubulointerstitium, which promotes renal fibrosis. The pathophysiological mechanisms that drive this process, including activation of the renin–angiotensin–aldosterone system (RAAS), decreased glomerular filtration rate, abnormal fluid and electrolyte balance, increased blood pressure, increased renal sympathetic nerve activity and inflammatory processes, have all linked to some extent to an imbalance between NO and ROS. Damage to the tubulointerstitium can stimulate the production of ROS, which may exacerbate endothelial cell injury, capillary reduction, chronic inflammation and the induction of a hypoxic environment. These factors can accelerate the progression of chronic kidney disease (CKD). **b**, Damage to endothelial cells in the glomerulus and peritubular capillary can induce injury in adjacent epithelial cells through imbalances in ROS/NO, which can lead to the activation of inflammatory and fibrotic pathways involving nuclear factor-κB (NF-κB), inflammasome activation and Wnt–β-catenin, resulting in kidney fibrosis. AKD, acute kidney disease; AKI, acute kidney injury; eNOS, endothelial nitric oxide synthase; NOX, NADPH oxidase; SASP, senescence-associated secretory phenotype.

antifibrotic effects of sGC stimulators and activators are mediated by cGMP, which inhibits the TGFβ-phospho-SMAD3 signalling pathway[15]. A phase II trial of the sGC stimulator, praliciguat, in patients with type 2 diabetes and albuminuria already receiving a RAS blocker did not meet its primary end point of a reduction in albuminuria. However, exploratory end points, including reductions in metabolic variables such as blood pressure, haemoglobin A1c, and cholesterol, favoured praliciguat treatment[219].

Pyridoxamine dihydrochloride

Clinical trials are also underway to study the effects of antioxidants in patients with kidney disease. Pyridoxamine dihydrochloride (pyridoline), a derivative of vitamin B_6, is considered a potential treatment option due to its ability to scavenge ROS and inhibit the formation of advanced glycation end products[220]. However, a double-blind, randomized, placebo-controlled study in patients with type 2 diabetes and proteinuric kidney disease failed to demonstrate an effect of pyridoline

Table 2 | Clinical trials targeting ROS pathways in kidney disease

Disease	Trial phase	Inclusion criteria	Number of patients enrolled	Drug	Status	Result	Clinical trial number or reference
AKI and AKI to CKD	I/II	Patients aged ≥18 years undergoing elective cardiac or aortic surgery with cardiopulmonary bypass; stable preoperative renal function, without dialysis	217	NO	Completed	Decreased incidence of AKI, and transition to stage 3 CKD	Lei et al. (2018)[214]
AKI and AKI to CKD	III	Patients aged ≥18 years undergoing elective cardiac or aortic surgery with cardiopulmonary bypass >90 min; stable preoperative renal function and without RRT; clinical evidence of endothelial dysfunction	250	NO	Active, not recruiting	No study results posted	NCT02836899
DKD in T2DM	II	Patients age 25–75 years with eGFR 30–75 ml/min/1.73 m^2 and UACR 200–5,000 mg/g	156	IW-1973 (sGC stimulator)	Completed	Treatment for 12 weeks did not significantly reduce albuminuria compared with placebo in the primary efficacy analysis	Hanrahan et al. (2020)[219]
DKD in T2DM	II	Patients aged ≥25 years with sCr 1.3–3.3 mg/dl (women) or 1.5–3.5 mg/dl (men), and a 24-h urine collection PCR ≥1,200 mg/g while on an ACEI or an ARB	317	Pyridoxamine dihydrochloride	Completed	No study results posted	NCT00734253
DKD in T2DM	III	Patients aged ≥18 years with sCr ≥1.25 mg/dl (women) or ≥1.45 mg/dl (men) and a 24-h urine collection PCR ≥1,200 mg/g	328	Pyridorin (pyridoxamine dihydrochloride)	Terminated	No study results posted	NCT02156843
DKD in T2DM	II	Patients aged 18–80 years UACR 300–3,500 mg/g; eGFR ≥30 ml/min/1.73 m^2, receiving an ACEI or an ARB	200	GKT137831 (a Nox1/4 inhibitor)	Completed	No study results posted	NCT02010242
DKD in T1DM	II	Patients aged 18–70 years with eGFR ≥40 ml/min/1.73 m^2 and UACR ≥2.5 mg/mmol (men) or ≥3.5 mg/mmol (women) receiving an ACEI or an ARB	92	GKT137831 (a Nox1/4 inhibitor)	Completed	No study results posted	ACTRN12617001187336
T2DM and CKD	II	Patients aged 18–80 years with UACR 200–3,000 mg/g and eGFR between 30 and 90 ml/min/1.73 m^2 receiving an ACEI or ARB	140	APX-115 (a pan NOX inhibitor)	Completed	No study results posted	NCT04534439
Contrast-induced AKI	II	Patients aged ≥18 years and eGFR >30 ml/min/1.73 m^2 and <90 ml/min/1.73 m^2	280	APX-115 (a pan NOX inhibitor)	Not yet recruiting	No study results posted	NCT05758896
DKD in T2DM	II	Patients aged 18–75 years with stage 3a CKD (eGFR 45 to <60 ml/min, UACR ≥600 mg/g), stage 3b CKD (eGFR 30 to <45 ml/min, UACR ≥300 mg/g) or stage 4 CKD (eGFR 15 to <30 ml/min, UACR ≥150 mg/g) receiving an ACEI or an ARB	334	Selonsertib	Completed	Selonsertib may delay the progression of DKD	NCT02177786
DKD in T2DM	II	Patients aged 18–80 years with eGFR ≥45 to <60 ml/min/1.73 m^2 and UACR 600–5,000 mg/g, eGFR ≥30 to <45 ml/min/1.73 m^2 and UACR 300–5, 000 mg/g, or eGFR ≥20 to <30 mL/min/1.73 m^2 and UACR ≥150–5,000 mg/g while receiving an ACEI or ARB	384	Selonsertib	Completed	Selonsertib may delay the progression of DKD	NCT04026165

ACEI, angiotensin-converting enzyme inhibitor; AKI, acute kidney injury; ARB, angiotensin receptor blocker; CKD, chronic kidney disease; DKD, diabetic kidney disease; eGFR, estimated glomerular filtration rate; NO, nitric oxide; NOX, NADPH oxidase; PCR, protein to creatinine ratio; RRT, renal replacement therapy; sCr, serum creatinine (multiply by 88.4 to obtain value in micromoles per litre); sGC, soluble guanylyl cyclase; T1DM, type 1 diabetes mellitus; T2DM, type 2 diabetes mellitus; UACR, urine albumin to creatinine ratio.

on serum creatinine levels. In addition, the phase III PIONEER study (NCT02156843) of pyridolide in patients with DKD was conducted. However, the results have not yet been published[221].

NOX1/4 inhibitors

Clinical trials are also underway to assess inhibitors of the NOX family of ROS-producing enzymes. The NOX1/4 inhibitor, GKT137831, has shown promising renoprotective effects in multiple animal models of CKD[222], leading to the initiation of clinical trials. A multicentre, randomized, placebo-controlled study of GKT137831 in patients with type 1 diabetes and kidney disease is currently underway[223]. In another phase II trial, isuzinaxib (APX-115), a novel pan NOX inhibitor, was found to significantly reduce albuminuria and to be safe and well-tolerated in patients with type 2 diabetes and CKD[224]. A further study is underway to assess the ability of isuzinaxib to prevent contrast-induced AKI in patients undergoing percutaneous coronary intervention (NCT05758896)[225].

ASK1 inhibitors

The ASK (apoptosis signal-regulating kinase) family of enzymes is activated in response to various stressors, including oxidative stress and high glucose levels. ASK1 is activated by ROS and advanced glycation end products, leading to the activation of the p38 MAPK signalling pathway and kidney fibrosis[226]. *Ask1*-knockout mice are protected from IRI and UUO-induced kidney injury, in association with suppressed p38 MAPK and c-Jun N-terminal kinase signalling[227,228]. The ASK1 inhibitor, GS-444217, also improved pathological features of DKD, such as albuminuria, glomerulosclerosis, podocyte loss and tubulointerstitial fibrosis in db/db eNOS$^{-/-}$ mice. Renal biopsy tissue from patients with diabetes shows evidence of ASK1 activation[229].

A phase II clinical trial of the ASK1 inhibitor, selonsertib, in 333 adults with moderate to advanced type 2 diabetes and CKD who did not respond to standard treatment did not meet its primary end point of change from baseline estimated glomerular filtration rate (eGFR) after 48 weeks. However, an exploratory post hoc analysis suggested that selonsertib may delay the progression of DKD[230].

Nrf2 regulators

Bardoxolone methyl is a synthetic triterpenoid compound that was developed as a potential therapeutic agent for malignant tumours. It is thought to inhibit the NF-κB pathway and to activate the Nrf2 transcription factor, which as described earlier, has a crucial role in regulating defence mechanisms against oxidative stress in the kidney. It is currently in clinical trials to evaluate its efficacy in various kidney diseases (Table 3).

Table 3 | Human trials of bardoxolone methyl in kidney disease

Trial	Trial number or reference	Aetiology	Baseline kidney function	Number of patients enrolled	Dose (mg per day)	Recruitment status	Outcome
BEAM	235	DKD	eGFR 20–45 ml/min/1.73 m^2	227	25–100	Completed	Increase in mean eGFR: 8.2±1.5 ml/min/1.73 m^2 (25-mg group), 11.4±1.5 ml/min/1.73 m^2 (75-mg group) and 10.4±1.5 ml/min/1.73 m^2 (100-mg group) at 24 weeks
MERLIN	NCT04702997	CKD	eGFR ≥20 to <60 ml/min/1.73 m^2 and UACR ≥300 mg/g; or eGFR decline at a rate of ≥4 ml/min/1.73 m^2 in prior year; or haematuria defined as >5–10 RBCs per high power field	81	5–30	Completed	The results of this trial are available on the ClinicalTrials.gov website. There are no published results.
TSUBAKI	237	DKD	eGFR ≥30 to <60 ml/min/1.73 m^2 and UACR <300 mg/g; or eGFR ≥15 to <30 ml/min/1.73 m^2 and UACR <2,000 mg/g	120	5–15	Completed	Significant improvement in GFR as measured by inulin clearance 6.64 ml/min/1.73 m^2; P=0.008 at 16 weeks
PHOENIX	NCT03366337	T1DM, IgAN, FSGS and ADPKD	eGFR between ≥30 and ≤90 ml/min/1.73 m^2 and UACR ≤2,500 mg/g	103	5–30	Completed	The results of this trial are available on the ClinicalTrials.gov website. There are no published results.
CARDINAL (phase II/III)	NCT03019185	Alport syndrome	eGFR 30–90 ml/min/1.73 m^2 and UACR ≤3,500 mg/g	157	5–30	Completed	Preservation of eGFR relative to placebo after a 2-year study period
BEACON	NCT01351675	DKD	eGFR between ≥15.0 and <30.0 ml/min/1.73 m^2	2,185	20	Terminated	Increase in eGFR of 5.5±0.2 in 9 months
AYAME	NCT03550443	DKD	eGFR between ≥15 to <30 ml/min/1.73 m^2 and UACR ≤3,500 mg/g	1,013	5–15	Terminated	Not published
FALCON	NCT03918447	ADPKD	eGFR ≥30 to ≤90 ml/min/1.73 m^2 and UACR ≤2,500 mg/g	850 (estimated)	5–30	Terminated	Not published

ADPKD, autosomal dominant polycystic kidney disease; CKD, chronic kidney disease; DKD, diabetic kidney disease; eGFR, estimated glomerular filtration rate; FSGS, focal segmental glomerulosclerosis; GFR, glomerular filtration rate; IgAN, IgA nephropathy; RBCs, red blood cells; T1DM, type 1 diabetes mellitus; UACR, urine albumin to creatinine ratio.

Itaconate is a metabolite that activates NRF2 by alkylating a critical cysteine residue in the KEAP1 protein, which blocks KEAP1-dependent proteolysis of NRF2 (refs. 231,232). Itaconate derivatives may therefore be useful in the treatment of inflammatory diseases and may also hold promise for the treatment of kidney disease. In an experimental model of sepsis, pretreatment of mice with 4-octyl itaconate (4-OI), a cell-permeable derivative of endogenous itaconate, alleviated lung injury and inhibited lipid peroxidation and injury of THP1 macrophages. Mechanistically, 4-OI inhibited ferroptosis by promoting the accumulation and activation of Nrf2 (ref. 233).

Bardoxolone methyl for the treatment of DKD. The improvement in eGFR levels in a phase I trial of patients with malignancy[234] was the driving force behind the initiation of a clinical trial in patients with type 2 diabetes mellitus with CKD. In the phase II BEAM study in patients with type 2 diabetes and CKD, patients receiving bardoxolone methyl had a significant increase in mean eGFR at week 24 compared to placebo, and this increase was maintained through week 52 (ref. 235). However, the subsequent phase III BEACON trial[236], was terminated early, after a median follow-up of 9 months, due to a higher incidence of cardiovascular events in the bardoxolone methyl group. At the time of termination, the incidence of the primary end point (a composite of kidney failure or death from cardiovascular causes) was similar in both the bardoxolone methyl group (69 of 1,088; 6%) and the placebo group (69 of 1,097; 6%), indicating no significant difference, despite a significant increase in eGFR, blood pressure and urinary albumin to creatinine ratio in the bardoxolone methyl group[236]. The phase II TSUBAKI study enrolled patients with stage 3 and 4 CKD and type 2 diabetes in Japan[237], and importantly excluded participants with a history of heart failure, or a brain natriuretic peptide (BNP) level greater than 200 pg/ml. After 16 weeks of treatment, the bardoxolone methyl group showed an increase in GFR compared to baseline (6.64 ml/min/1.73 m^2 increase), and the drug was well tolerated with no evidence of fluid retention. The phase III AYAME study was conducted to further investigate the effect of bardoxolone methyl on CKD in patients with CKD stages 3 and 4. Results have not yet been reported[238].

Bardoxolone methyl for the treatment of Alport syndrome. Alport syndrome is the second most common inherited cause of kidney failure. In the phase II/III CARDINAL study (NCT03019185)[239] treatment with bardoxolone methyl on top of standard therapy resulted in preservation of eGFR among adolescent and adult patients with Alport syndrome compared with placebo at 48 and 100 weeks after randomization. However, this benefit disappeared after treatment discontinuation[240].

Bardoxolone methyl for the treatment of autosomal dominant polycystic kidney disease. The FALCON study (NCT03918447)[241] is a multinational, placebo-controlled phase III clinical trial that aims to evaluate the safety and efficacy of bardoxolone methyl in patients with autosomal dominant polycystic kidney disease (ADPKD). The study has two primary end points, including changes in the eGFR at week 108 and the number of adverse events reported during the 112-week study period. The EAGLE study (NCT03749447)[242] is an ongoing, international, multicentre, open-label study, that aims to provide expanded access to patients enrolled in the CARDINAL and FALCON studies. The primary aim of the study is to assess alterations in eGFR after 12 weeks in individuals who have received the initial dose of the drug.

Bardoxolone methyl for treatment of progressive and rare forms of CKD. The MERLIN study (NCT04702997)[243] is a multicentre, placebo-controlled, phase II trial that aims to investigate the use of bardoxolone methyl in patients with CKD who are at risk of rapid kidney function decline. The PHOENIX study (NCT03366337) is a phase II clinical trial[216] that aims to assess the safety, tolerability and efficacy of bardoxolone methyl in patients with rare forms of CKD, including that associated with type 1 diabetes, IgA nephropathy, focal segmental glomerulosclerosis and ADPKD.

Conclusions

Oxygen is essential for life; however, it can also produce ROS, which at excess levels can adversely affect lipids, proteins, DNA and other cellular components. Maintaining a balance between ROS production and antioxidant consumption is crucial for maintaining redox balance. Insufficient levels of antioxidants or the excess production of ROS induces a state of oxidative stress. The imbalance between the production and consumption of ROS can be caused by various factors, including lifestyle and environmental factors, underlying health conditions and the natural ageing process, and can exacerbate disease conditions, including CKD. Insights into the mechanisms by which ROS are produced, the factors that contribute to their balance, and their homeostatic and pathogenic functions will be necessary to identify specific and safe therapeutic targets in CKD and other diseases that are associated with oxidative stress.

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Author contributions

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Competing interests

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Additional information

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