Review Article

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KIDNEY RESEARCH AND CLINICAL PRACTICE

The role of oxidative stress and hypoxia in renal disease

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Oxygen is required to sustain aerobic organisms. Reactive oxygen species (ROS) are constantly released during mitochondrial oxygen consumption for energy production. Any imbalance between ROS production and its scavenger system induces oxidative stress. Oxidative stress, a critical contributor to tissue damage, is well-known to be associated with various diseases. The kidney is susceptible to hypoxia, and renal hypoxia is a common final pathway to end stage kidney disease, regardless of the underlying cause. Renal hypoxia aggravates oxidative stress, and elevated oxidative stress, in turn, exacerbates renal hypoxia. Oxidative stress is also enhanced in chronic kidney disease, especially diabetic kidney disease, through various mechanisms. Thus, the vicious cycle between oxidative stress and renal hypoxia critically contributes to the progression of renal injury. This review examines recent evidence connecting chronic hypoxia and oxidative stress in renal disease and subsequently describes several promising therapeutic approaches against oxidative stress.

Keywords: Hypoxia, Hypoxia-inducible factor, Nuclear factor erythroid 2-related factor 2, Oxidative stress, Renal insufficiency, chronic

Introduction

Oxygen is an essential molecule for the sustenance of aerobic organisms. Reactive oxygen species (ROS), including superoxide radicals ($O_2^{\bullet -}$), hydrogen peroxide (H_2O_2), hydroxyl radicals ($^{\bullet}OH$), and singlet oxygen ($^1O_2$), are constantly released when oxygen is consumed to produce adenosine triphosphate (ATP). ROS are harmful to cells and tissues, so specific biological systems remove and detoxify them. A proper balance between ROS production and removal is required to maintain normal biological conditions. Oxidative stress is defined as a ROS level increase and the damage it causes. It is caused by an imbalance between ROS production and removal and is associated with a variety of diseases, such as cancers, cardiovascular diseases, neurological diseases, and infertility [1–5]. The concept of oxidative stress and its components are well known, and recent successes with ROS-oriented therapeutic approaches have attracted the attention of both basic and clinical researchers. Persistent oxidative stress is known to play a crucial role in the pathogenesis of acute kidney injury (AKI) and to be involved in the AKI-to-chronic kidney disease (CKD) transition [6,7].

Because oxygen has an oxidative effect, hyperoxia causes oxidative stress. In clinical settings, oxygen therapy must only be administered to patients for whom an oxygen supplement is safe because hyperoxia often causes lung injury [8]. Hypoxia, paradoxically, also induces oxidative stress through increased ROS production, superoxide radicals ($O_2^{\bullet -}$) in particular, by oxygen-dependent enzymes such

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as cytochrome c oxidase, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase, and uncoupled endothelial nitric oxide synthase [9]. Given that the kidney is the organ most susceptible to hypoxia and that renal hypoxia usually occurs during kidney disease progression, as shown below, hypoxia, and not hyperoxia, must play an important role in the aggravation of oxidative stress in CKD [10–13]. This review examines recent evidence for the pathogenic role of chronic hypoxia and oxidative stress in renal diseases and describes promising therapeutic approaches against oxidative stress.

Unique oxygen dynamics of the kidney

Although renal blood flow accounts for as much as 20% of the cardiac output, renal tissue oxygen tensions are comparatively low due to oxygen shunt diffusion between the arterial and venous vessels, which run parallel to each other in the kidney [10]. For example, the partial pressure of oxygen was reported to be higher in the renal vein than at any site in the efferent arterioles or the cortex, which implies precapillary oxygen shunting from the arteries to the veins inside the kidney [14]. The existence of a decreasing oxygen gradient with respect to the depth from the renal surface (i.e., 10–20 mmHg in the renal medulla vs. 50 mmHg in the renal cortex), also supports the presence of oxygen shunt diffusion because renal arterial flow spreads from the renal pedicle to the renal surface [15]. This oxygen shunt makes the kidney the organ most susceptible to systemic hypoxia; microvascular oxygen pressure in the kidney starts to decrease with a smaller hematocrit drop than required for other organs, even in normal kidneys [16]. In CKD, tubulointerstitial hypoxia is caused by multifactorial mechanisms: loss of peritubular capillaries, decreased oxygen diffusion from the peritubular capillaries to tubular and interstitial cells as a result of fibrosis, stagnation of peritubular capillary blood flow induced by sclerosis of 'parent' glomeruli, decreased peritubular capillary blood flow as a result of an imbalance of the vasoactive substances, increased metabolic demands of tubular cells, inappropriate energy usage as a result of uncoupled mitochondrial respiration induced by oxidative stress, and decreased oxygen delivery as a result of anemia [11]. Therefore, chronic tubulointerstitial hypoxia occurs in parallel with CKD progression, and it has been assumed that chronic tubulointerstitial hypoxia is a common final step in the development of end-stage renal disease (ESRD) from a variety of causes [11].

Measurement of oxygen status in the kidney

Investigating tubulointerstitial hypoxia requires an ability to measure the oxygen status in the kidney. Several methods have been developed to evaluate that oxygen status, and each of them has advantages and disadvantages (Table 1). In animal experiments, the use of microelectrodes has been the gold standard for quantitative oxygen tension measurement in solid organs. The oxygen tension of the cortex and medulla of a normal kidney, measured by microelectrodes, was reported to be approximately 50 mmHg and 30 mmHg, respectively [17]. This method can be applied to models of kidney diseases such as ischemia/reperfusion injury and polycystic kidney disease [18,19]. It also has disadvantages: it is limited to extracellular regions, it is difficult to use in clinical applications because of its invasiveness, and the oxygen tension and renal flow results might be affected by renal decapsulation [20]. Therefore, other hypoxia detection methods are currently under development: blood oxygen level–dependent magnetic resonance imaging (BOLD-MRI), nitroimidazole probes, and phosphorescence lifetime measurement [17].

BOLD-MRI measures the deoxyhemoglobin concentration in veins and is a non-invasive technique. Though it has been tested in clinical trials, studies using BOLD-MRI in CKD patients to examine the relationship between

Table 1. Different methods of measuring oxygen status in the kidney

Method	Quantitative	Intracellular	Extracellular	Clinical use
Microelectrodes	Yes	No	Yes	No
BOLD-MRI	Yes	No	Yes (vein)	Yes
Nitroimidazole probes	No	Yes	No	Yes (PET tracers)
Phosphorescent lifetime measurement	Yes	Yes	Yes	No

BOLD-MRI, blood oxygen level–dependent magnetic resonance imaging; PET, positron emission tomography.

renal prognosis and renal hypoxia have shown conflicting results [21,22]. Recently, a more precise renal BOLD-MRI value, the twelve-layer concentric objects method, was developed [23] to expand the potential to use renal hypoxia detected by BOLD-MRI as a progression marker for CKD [24]. However, only venous oxygen status can be assessed by BOLD-MRI. Although intravenous oxygen status is important, oxygen status in other compartments, such as intraarterial or intracellular oxygen status, are important as well. Nitroimidazole probes are frequently used as intracellular hypoxic markers. Pimonidazole, a 2-nitroimidazole hypoxic marker, is reductively activated in an oxygen-dependent manner and is covalently bound to thiol-containing proteins in hypoxic cells. Intracellular hypoxia (below 10 mmHg of oxygen partial pressure) can be detected immunohistochemically by pimonidazole–protein adducts. Several positron emission tomography tracers with nitroimidazoles have been studied for clinical applications [17,25,26]. Another promising method is phosphorescence lifetime measurement, which can visualize intravital oxygen status and quantify intracellular oxygen concentrations, which are otherwise difficult to do. The phosphorescent dye, which is based on an iridium (III) complex, (benzothienylpyridine) 2Ir (acetylacetone) with a cationic dimethylamino group, enabled the measurement of intracellular oxygen tension [27]. This technique was applied to mouse kidneys and successfully measured the intracellular partial pressures of oxygen in tubular cells [28]. In that study, ischemia/reperfusion models showed that oxygen pressure dropped at the ischemia phase and subsequently recovered at the reperfusion phase [28]. Afterward, the phosphorescence lifetime imaging technique was established and showed the presence of an oxygen gradient in the surface of the cortex, even in normal mouse kidneys [29]. This method could also be expanded to assess oxygen levels in other compartments using different phosphorescent probes. The biggest limitation of this technique is its need for special instruments. Precise understanding and complementary use of these different methods are crucial for the accurate assessment of renal oxygenation.

Importance of hypoxia in CKD and the AKI-to-CKD transition

According to the International Society of Nephrology, 10% of the world's population is affected by CKD, and millions die from it every year [30]. As indicated by its severe effects on public health, renal hypoxia, a common prognostic factor in a variety of kidney diseases, is certainly an important challenge.

CKD-associated pathologic mechanisms are varied: changes in signaling pathways, mitochondrial dysfunction and oxidative stress, changes in autophagy, chronic inflammation, vascular dysfunction, and epigenetic regulation [31]. An accumulation of basic and clinical research has established that hypoxia-induced renal fibrosis is a common pathological hallmark of CKD. CKD aggravates tubulointerstitial chronic hypoxia through the multifactorial causes listed above [11]. Renal hypoxia, in turn, accelerates CKD progression. That is, renal hypoxia is both the cause and consequence of kidney disease [32]. Its vicious cycle contributes to CKD progression.

Renal hypoxia is associated not only with CKD but also with the AKI-to-CKD continuum. Although renal function can recover, AKI often results in the development of CKD. Observational studies report that 20% to 50% of AKI survivors develop CKD. From a pathological perspective, incomplete or maladaptive repair after AKI results in kidney fibrosis, which develops and progresses to CKD. Recent studies show that tubulointerstitial hypoxia is a key player in the pathophysiology of the AKI-to-CKD transition and CKD [33]. Capillary rarefaction after AKI episodes induces renal hypoxia, which can affect tubular epithelial cells, fibroblasts, and inflammatory cells, resulting in tubulointerstitial fibrosis. Damaged tubular epithelial cells that fail to re-differentiate might supply a decreased amount of vascular endothelial growth factor (VEGF) and contribute to capillary rarefaction, thereby aggravating hypoxia and forming a vicious cycle [33,34]. Other studies have demonstrated that renal hypoxia induces diverse epigenetic changes such as DNA methylation, histone modification, and chromosome conformational changes, and those epigenetic changes are stored in cells as "hypoxic memory," which can induce the AKI-to-CKD transition as a long-term effect [7,35]. Among elderly people, AKI frequently occurs in CKD patients. AKI on top of CKD has poor renal prognosis because CKD-associated pathological changes can increase renal sensitivity or susceptibility to AKI and suppress kidney repair or recovery from AKI in CKD patients [31].

Oxidative stress in CKD

Oxidative stress caused by an imbalance between ROS production and scavenging occurs through a dysfunction of mitochondrial respiration. Mitochondrial dysfunction can be caused by renal damage that stems from multiple factors, including aging, diabetes mellitus, and inflammation.

Oxidative stress is enhanced in CKD patients, especially those with diabetic kidney disease (DKD). It is well-known that chronic hyperglycemia induces ROS production in diabetes [36]. ROS-mediated renal inflammation and renal fibrosis contribute to the pathology of DKD through multiple signaling pathways that involve transforming growth factor-β, monocyte chemoattractant protein-1, connective tissue growth factor, tumor necrosis factor-α, interleukin (IL) 1, IL-6, IL-18, and cell adhesion molecules [12].

Several markers related to oxidative stress, such as 8-hydroxy-2′-deoxyguanosine (8-OHdG), malondialdehyde (MDA), and advanced glycation end products (AGEs), have been used to predict renal prognosis. 8-OHdG is a product of oxidative DNA damage, and 8-OHdG excreted in urine has been widely used as a non-invasive systemic oxidative stress marker in both animal experiments and clinical research. Recent studies have demonstrated that urinary 8-OHdG reflects an increased systemic level of oxidative DNA damage in DKD patients and the severity of DKD [37,38]. A high serum concentration of MDA, a lipid peroxidation marker, is found in uremic patients. An increased plasma level of MDA could be an early prognostic indicator of graft dysfunction after kidney transplantation [39]. AGEs are formed during glycation and oxidative stress, and therefore, an increase in oxidative stress results in increased AGEs, which explain the accumulation of AGEs in CKD patients who are not diabetic. Therefore, AGEs are uremic toxins. AGE deposition in skin, reflecting systemic AGE accumulation, can be noninvasively measured by skin autofluorescence, and is predicted to become a novel risk marker for CKD [40–42]. The utility of these oxidative stress–induced prognostic biomarkers corroborates the importance of oxidative stress in CKD patients. The best predictor for disease prognosis has not yet been identified. 8-OHdG differs substantially among individuals because it is affected by smoking, exercise, and dietary habits, and MDA results depend on the detection method used [43]. Although the effectiveness of skin autofluorescence in measuring AGEs has been shown in many previous reports on renal and cardiovascular diseases, AGEs do not reflect only oxidative stress [44].

Biological response to hypoxia and oxidative stress

Chronic renal hypoxia is certainly present in CKD. However, living organisms are often exposed to hypoxia for a variety of reasons, such as ischemia, hypoxemia, and anemia. Therefore, a defense mechanism against hypoxia called the hypoxia-inducible factor (HIF) pathway evolved. In hypoxic conditions, HIF accumulates due to decreased HIF hydroxylation by prolyl hydroxylase (PHD), a rate-limiting enzyme in HIF degradation that requires an oxygen molecule as a substrate. HIF activation promotes the expression of downstream genes such as glucose transporter 1 (*GLUT1*), erythropoietin (*EPO*), and *VEGF*, which counteract hypoxia by increasing glucose uptake, erythropoiesis, and angiogenesis, respectively [11]. Another function of HIF activation is oxidative stress reduction by the inhibition of ROS production and the enhancement of detoxification. An example of inhibiting ROS production is mitochondrial *NDUFA4L2* (NADH dehydrogenase [ubiquinone] 1 alpha subcomplex, 4-like 2) expression, which is induced by hypoxia. *NDUFA4L2* expression is upregulated by HIF, which attenuates mitochondrial oxygen consumption by inhibiting the activity of complex I (NADH: ubiquinone oxidoreductase) in the electron transport chain to limit intracellular ROS production under low-oxygen conditions [45]. Another study showed that HIF optimized the efficiency of respiration by altering the composition of a cytochrome c oxidase subunit, resulting in decreased ROS and increased ATP production [46]. Heme oxygenase 1 (HO-1) and superoxide dismutase 1 (SOD-1) are well-known ROS-detoxifying enzymes regulated by HIF [47–49].

A master regulator of defense responses to oxidative stress is the nuclear factor erythroid 2-related factor 2 (Nrf2)–Kelch-like ECH-associated protein 1 (KEAP1) interaction. In normal conditions, KEAP1 binds to Nrf2, resulting in proteasomal degradation of Nrf2. Under oxidative stress, the molecular structure of KEAP1 changes until it loses the ability to bind Nrf2. The resulting accumulation of Nrf2 leads to Nrf2 translocation to the nu-

cleus, which promotes the expression of a variety of cytoprotective genes related to redox and detoxification [50]. On the other hand, p38 mitogen-activated protein kinase (MAPK) and c-Jun amino terminal kinase (JNK) signaling are upregulated under oxidative stress, and they are associated with cell death and inflammation [50,51].

The effectiveness of activating the HIF pathway or Nrf2 and inhibiting apoptosis signal-regulating kinase 1 (ASK-1), an upstream signaling kinase of p38 MAPK and JNK, in the treatment of renal diseases is now under active investigation to enable future clinical applications of antioxidative treatments, as described in detail below.

Link between hypoxia and oxidative stress in CKD

The significance of hypoxia and oxidative stress in CKD has been described above. There is an intricate link between renal hypoxia and oxidative stress.

Oxidative stress is enhanced in CKD, especially in DKD [36]. Increased oxidative stress causes increased kidney oxygen consumption, which results in kidney tissue hypoxia. An underlying mechanism is the accumulation of uremic toxin. Indoxyl sulfate (IS), a representative uremic toxin, causes oxidative stress, which results in increased oxygen consumption and hypoxia [52]. Other uremic toxins, such as phenyl sulfate and ρ-cresyl sulfate, enhance tubular cell susceptibility to oxidative stress by depleting the glutathione level. Hyperuricemia is another mechanism that causes increased oxidative stress in CKD. Long-term hyperuricemia results in increased renal oxidative stress and mitochondrial dysfunction [53]. These findings indicate that oxidative stress induced by uremic toxins in CKD aggravates renal hypoxia.

Renal hypoxia, in turn, magnifies renal oxidative stress. Oxidative stress caused by excess ROS production generally leads to renal inflammation and fibrosis via diverse signaling pathways [12]. It is certain that hypoxia and hyperoxia both result in mitochondrial generation of ROS in various organs, including the kidney. To explain the paradoxical increase in ROS production during hypoxia, an interesting experiment demonstrated that ROS generated by complex III of the electron transport chain stabilized HIF, which implies that mitochondria have a potential oxygen sensing mechanism at complex III. The discovery of the molecules that act as oxygen sensors will provide a therapeutic strategy for oxidative stress [54]. A study that used dinitrophenol, a mitochondrial uncoupler that increases oxygen consumption, found that kidney tissue hypoxia, *per se*, without confounding hyperglycemia or oxidative stress, could be sufficient to initiate the development of nephropathy [55].

Hypoxia and oxidative stress are intricately linked, and both aggravate the progression of CKD. Therefore, treatments against hypoxia and oxidative stress are needed to dissolve this vicious cycle.

Traditional treatments targeting oxidative stress

Angiotensin II type 1 receptor blockers (ARBs)

Proteinuria and hypertension correlate closely with renal prognosis. Evidence has accumulated that ARBs have renoprotective effects, including decreased proteinuria independent of blood pressure lowering. The beneficial effects of ARBs on DKD have been supported in several clinical trials, such as the Reduction of Endpoints in non-insulin-dependent diabetes with the Angiotensin II Antagonist Losartan study and the Irbesartan Diabetic Nephropathy Trial [56,57].

The constriction of efferent arterioles by angiotensin II, with an attendant decrease in peritubular capillary flow, results in renal hypoxia. The main renoprotective mechanism of the ARBs is thus blocking the constriction of efferent arterioles by angiotensin II [58]. Basic research comparing several kinds of antihypertensive agents showed that ARBs had additional renoprotective properties by decreasing oxidative stress and inhibiting AGE formation and abnormal iron deposition [58].

N-acetylcysteine (NAC)

The beneficial effects of NAC in renal diseases are unclear.

NAC, an acetylated variant of amino acid L-cysteine, is a precursor for glutathione synthesis, which works as a direct scavenger of free radicals. NAC can also improve blood flow through nitric oxide–mediated vasodilation. Both the antioxidant and vasodilatory properties of NAC are important to its use as a therapeutic agent for CKD. A population-based cohort study of CKD patients showed that NAC use was associated with a reduced risk of progression to dialysis [59]. Randomized controlled trials

demonstrated that high-dose statins plus hydration with NAC decreased contrast-induced nephropathy (CIN) [60,61].

On the other hand, several reports have denied the beneficial effects of NAC on renal diseases. In a mouse ischemia-reperfusion model, NAC enhanced the progression to CKD following AKI, not only by dampening endogenous cellular antioxidant responses at the time of injury but also by enhancing persistent kidney mitochondrial and metabolic dysfunction [62]. According to a comprehensive study, NAC did not reduce CIN incidence in patients undergoing peripheral angiography [60,61].

The effects of NAC on renal diseases have been controversial, so further studies are required.

Antidiabetics

The probable renoprotective effects (oxidative stress attenuation in DKD) of several antidiabetics, including dipeptidyl peptidase-4 (DPP-4) inhibitors, glucagon-like peptide-1 (GLP-1) receptor agonists, and sodium glucose cotransporter-2 (SGLT-2) inhibitors, has been supported by several studies. Although a basic study demonstrated renal injury amelioration in DPP-4-deficient diabetic rats through a blockade of AGE receptor axis–induced oxidative stress, clinical evidence remains insufficient [63]. However, clinical evidence for the renoprotective effect of GLP-1 agonists was provided by the Liraglutide Effect and Action in Diabetes: Evaluation of Cardiovascular Outcome Results trial, in which the liraglutide group had a significantly better renal outcome, defined as a composite of new-onset persistent macroalbuminuria, persistent serum creatinine level doubling, ESRD, and renal disease-related death, than the placebo group [64]. Animal experiments have shown that the renoprotective effects of GLP-1 agonists are independent of their glucose-lowering action. GLP-1 binds to its receptors on glomerular capillary and vascular walls, which upregulates cyclic adenosine monophosphate (a major messenger) and activates protein kinase A, possibly ameliorating oxidative renal injury by inhibiting a major source of superoxide anions [65,66]. Moreover, SGLT-2 inhibition is also expected to normalize increased oxidative stress in diabetic kidneys; imaging mass spectrometry revealed that SGLT-2 inhibition ameliorated oxidized glutathione accumulation in the kidney cortexes of diabetic mice [67]. Recently, a double-blind, randomized trial titled "Canagliflozin and Renal Events in Diabetes with Established Nephropathy Clinical Evaluation" showed that the relative risk of the primary outcome, a composite of ESRD, serum creatinine level doubling, and renal or cardiovascular disease-related death, was 30% lower in the canagliflozin group than in the placebo group [68].

Future treatments for renal oxidative stress

Nrf2 activator

Nrf2 is a critical transcription factor regulated by KEAP1, which binds to cytosolic Nrf2 and promotes its degradation by the proteasome system under basal conditions. Under oxidative stress, modification of the cysteine residues on KEAP1 limits the KEAP1–Nrf2 interaction, allowing Nrf2 translocation to the nucleus, which promotes the expression of a variety of cytoprotective genes related to redox and detoxification [50]. Bardoxolone methyl and itaconate derivatives are future therapeutic drug candidates for CKD that modulate the KEAP-NrF2 system.

Bardoxolone methyl was produced as an antioxidant inflammation modulator that worked by activating the KEAP1–Nrf2 pathway. Given that oxidative stress is particularly enhanced in diabetic patients, the renoprotective effect of bardoxolone methyl in CKD patients with diabetes has been investigated in several clinical trials.

In the BEAM randomized, placebo-controlled, 52-week trial of bardoxolone methyl treatment for renal function in CKD/type 2 diabetes patients, bardoxolone methyl was associated with improvement in the estimated glomerular filtration rate (eGFR) at 24 weeks, which was maintained for as long as 52 weeks [69]. In the Bardoxolone Methyl Evaluation in Patients with Chronic Kidney Disease and Type 2 Diabetes Mellitus: the Occurrence of Renal Events (BEACON) trial of patients with CKD and type 2 diabetes mellitus, the bardoxolone methyl group showed a significant mean increase in the eGFR from baseline values (5.5 mL per minute per 1.73 m^2) [70]. However, that study was prematurely terminated because of a higher rate of cardiovascular events that led to hospitalization or death due to heart failure in the bardoxolone methyl group than in the placebo group [70]. Although there is a hypothesis that bardoxolone methyl affects endothelin-signaling, the mechanism through which

bardoxolone methyl increased heart failure has not been adequately elucidated [71,72].

In the BEACON trial, 56% and 11% of patients had a history of cardiovascular disease and a history of hospitalization for heart failure, respectively. The high cardiovascular risk of the trial population might thus have resulted in an increased occurrence of cardiovascular diseases. Given the high public-health concerns about DKD, bardoxolone methyl continues to be developed in Japan, with careful attention paid to cardiac events. The Phase II Study of Bardoxolone Methyl in Patients with Chronic Kidney Disease and Type 2 Diabetes (TSUBAKI) trial, a randomized, double-blind, placebo-controlled, multicenter phase 2 trial in Japan, was conducted in patients with DKD who had no risk factors for volume overload or prior history of heart failure. It demonstrated significant improvement in GFR as measured by standard inulin clearance [73,74].

The AYAME study, an RTA 402 phase 3 clinical trial (a randomized, double-blind, placebo-controlled clinical trial in patients with DKD) has been initiated in Japan. This study is designed to assess the efficacy and safety of bardoxolone methyl in DKD [75]. In addition, a global phase 2/3 clinical study in patients with Alport's syndrome is currently being conducted [75]. Bardoxolone methyl is a novel promising therapeutic agent for CKD, but more evidence of its safety and efficacy is required.

A cell-permeable itaconate derivative, 4-octyl itaconate (OI), is an alternative activator of the KEAP1-Nrf2 system, which is also expected to be a novel treatment agent for CKD through oxidative stress reduction [73,76]. Further investigation of the clinical applications of OI is needed.

ASK-1 inhibitor

According to previous research, the stress-induced activation of p38 MAPK and JNK signaling is associated with the progression of kidney disease, and blockading that signaling can prevent renal inflammation and fibrosis and contribute to the improvement of renal prognosis [51].

ASK-1 is an upstream signaling kinase of p38 MAPK and JNK that is activated via autophosphorylation only in pathological states. It induces apoptotic, inflammatory, and fibrotic signaling under oxidative stress. Autophosphorylation of ASK-1 enhances the phosphorylation of p38 MAPK and JNK, which drives inflammation and fibrosis. It was shown that the ASK-1 pathway is activated in DKD patients and diabetic mice [77]. Recently, two ASK-1 inhibitors, GS-444217 and GS-4997, have been developed. They inhibit the autophosphorylation of ASK-1 and the subsequent inflammatory response. Several animal experiments have shown that GS-444217 had renoprotective effects [78,79]. One significant result was that GS-444217 reduced creatinine, blocked an increase in albuminuria, and reduced the pathological features of DKD such as glomerulosclerosis, loss of podocytes, tubulointerstitial fibrosis, and apoptosis in a diabetic mouse model. It also increased the efficacy of an ACE inhibitor on blood pressure reduction, decreased albuminuria, and regressed glomerulosclerosis in a rat 5/6 nephrectomy model [77]. A phase 2 clinical trial of GS-4997 (Selonsertib), another ASK-1 inhibitor, is now in progress in patients with DKD [73,80].

Vascular adhesion protein-1 (VAP-1) inhibitor

VAP-1 is a molecule implicated in various conditions associated with oxidative stress and inflammation. VAP-1 is an ectoenzyme that catalyzes the oxidative deamination of primary amines and generates H_2O_2, a source of oxidative stress, in the extracellular space. VAP-1 is expressed in the vascular endothelium of various tissues, including the kidney [81]. In a prospective cohort study done in Taiwan, high serum VAP-1 was found to be associated with the risk of ESRD [82], indicating that inhibition of VAP-1 might be a novel therapeutic approach against renal injury. According to the ALBUM trial, a randomized, double-blind, placebo-controlled phase 2 trial, an oral inhibitor of VAP-1 (ASP8232) in combination with an ACE inhibitor or ARB therapy effectively reduced albuminuria in participants with type 2 diabetes and an eGFR between 25 and 75 mL/min per 1.73 m^2 [81]. Another specific VAP-1 inhibitor, RTU-1096, exhibited a renoprotective effect in a renal ischemia/reperfusion model in rats by reducing neutrophil infiltration, generating a local hydrogen peroxide gradient [83]. Future studies on the effects of VAP-1 inhibitors are necessary.

Erythropoiesis-stimulating agents (ESAs)

Renal anemia is an important factor of increased oxida-

tive stress in CKD patients. ESAs are currently being used as standard therapy agents for the treatment of anemia in patients with CKD. Among many clinical trials examining the beneficial effects of anemia correction on CKD progression, several reports have demonstrated that treatment with an ESA reduces oxidative stress and could improve the prognosis for cardiovascular comorbidity and mortality in CKD patients [84]. The administration of alpha-darbepoetin rebalanced erythrocyte glutathione and its disulfide form in CKD patients treated with iron, which might have a clinical benefit on oxidative stress [84]. Another study investigating two major LPO products, 4-hydroxynonenal and MDA, in CKD patients undergoing dialysis suggested that the corresponding serum levels decreased during correction of renal anemia by epoetin [85]. These facts suggest that EPO could have antioxidative mechanisms. The direct antioxidative effects of EPO include an increase in HO-1 and other antioxidative enzymes. In addition, EPO indirectly exerts antioxidative effects by inducing body iron depletion, which reduces iron-dependent oxidative injury and causes an increase in the number of young blood cells loaded with substantial amounts of antioxidative enzymes, which increases the amount of circulating antioxidants [47].

HIF stabilizer

HIF activation promotes the expression of EPO and many other downstream genes. *HO-1* and *SOD-1* are downstream genes known for ROS-detoxification. The mitochondrial *NDUFA4L2*, another target gene of HIF, limits intracellular ROS production under hypoxia [45]. Thereby, HIF activation contributes to renal anemia correction and oxidative stress reduction by the inhibiting ROS production and enhancing detoxification. Therefore, HIF activation is expected to have a renoprotective effect. Indeed, accumulated evidence in animal experiments reveals that HIF activation and erythropoiesis have renoprotective effects. In streptozotocin-induced diabetic rats, treatment with chronic cobalt chloride, a traditional chemical stabilizer of HIF, prevented DKD via reduced oxidative stress [49]. The renoprotective effect of the HIF stabilizer was replicated in the 5/6th nephrectomy model [86]. However, cobalt chloride cannot be used clinically because of hepatic toxicity.

Recently, HIF stabilizers that can be clinically applied have been investigated. HIF is regulated by PHDs, which promote the degradation of HIF under normal oxygen conditions. PHDs are inhibited in hypoxic conditions, resulting in HIF accumulation as a defense against hypoxia. PHD inhibitors have been developed as novel oral HIF stabilizers, and five different oral agents, roxadustat, daprodustat, molidustat, vadadustat, and enarodustat, are now in phase 2 or phase 3 clinical trials investigating their effects on renal anemia [87]. Roxadustat (FG-4592) increased hemoglobin levels in non-dialysis CKD patients using neither intravenous iron nor ESA in a phase 2 study [88,89]. Vadadustat (AKB-6548) raised and maintained hemoglobin levels while enhancing iron mobilization via decreased hepcidin in CKD patients in a phase 2 study [90,91]. These oral PHD inhibitors require further investigation before they can be used clinically to replace ESA as a novel therapy for renal anemia. HIF activation stimulates a variety of downstream genes other than EPO, which means that the PHD inhibitors could have unpredictable systemic effects.

Reduction of IS

IS, a representative uremic toxin, accumulates in CKD patients and causes various cytotoxic effects in kidney proximal tubular cells [92]. Clinical studies have shown that IS accumulation has deleterious effects in renal disease. A potential vicious mechanism of IS includes the activation of NADPH oxidase 4, which causes an increase in ROS production. This direct IS-induced oxidative stress enhancement produces renal fibrosis. In addition, IS-induced anemia contributes to increased oxidative stress. Basic research demonstrated that IS suppressed HIF-dependent EPO production and led to renal anemia [93]. A recent work elucidated the mechanism of IS-induced activation of the aryl hydrocarbon receptor (AhR), a ligand-activated transcription factor that normally exists in the cytosol in an inactive form. IS increased the AhR-ARNT (AhR nuclear translocator) complex in the nucleus and subsequently suppressed nuclear accumulation of the HIF–ARNT complex, which reduced EPO production [94]. Therefore, IS reduction might be a therapeutic approach for renal anemia. Clinically, oral adsorbents that reduce IS, such as AST-120, have been investigated in clinical trials of CKD patients, including those undergoing dialysis [95]. Clinical evidence for the relationship

between IS and renal anemia is insufficient.

Conclusion

As explained in this review, oxidative stress and chronic hypoxia are intricately linked in CKD, and the discovery of therapeutic approaches against increased oxidative stress is in progress (Fig. 1, Table 2).

It has been well established that increased oxidative stress correlates with a variety of diseases in multiple organs. In the kidney, oxidative stress is upregulated in CKD. Given that the kidney is uniquely susceptible to hypoxia, renal hypoxia (not hyperoxia) is an important source of oxidative stress. Renal hypoxia increases oxidative stress, which in turn exacerbates renal hypoxia. The vicious cycle between oxidative stress and tissue hypoxia plays an important part in CKD progression. Therefore, novel therapeutic approaches against oxidative stress are needed for the treatment of renal diseases. Several promising agents, such Nrf2 activators and HIF stabilizers,

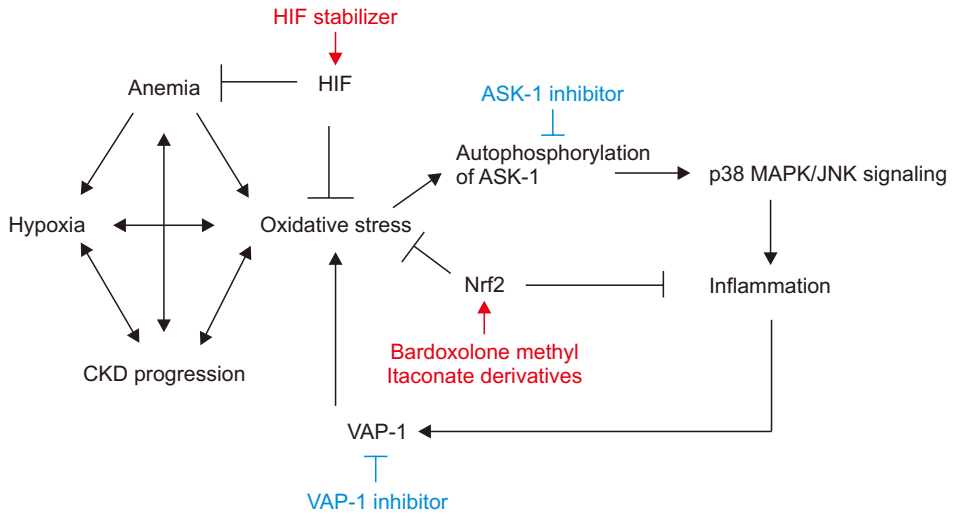

Figure 1. The link between hypoxia and oxidative stress in chronic kidney disease (CKD) and tools for intervention. Hypoxia and oxidative stress are intricately linked in CKD via diverse signaling pathways. Hypoxia-inducible factor (HIF) and nuclear factor erythroid 2-related factor 2 (Nrf2) activation reduce oxidative stress. Mitogen-activated protein kinase/c-Jun amino terminal kinase (p38 MAPK/JNK) signaling activated by increased oxidative stress upregulates inflammation. Vascular adhesion protein-1 (VAP-1) increases oxidative stress by generating inflammation; ASK-1, apoptosis signal-regulating kinase 1.

Table 2. Novel oxidative stress–focused drugs

Drug	Antioxidant mechanism	Clinical trials (including those in progress)
Nrf2 activator • Bardoxolone methyl • Itaconate derivatives	Activation of KEAP1-Nrf2 pathway	BEAM, BEACON, TSUBAKI, and AYAME trial
ASK-1 inhibitor	Suppression of autophosphorylation of ASK-1 and subsequent p38 MAPK and JNK signaling	A phase 2 clinical trial of GS-4997
VAP-1 inhibitor	Inhibition of hydrogen peroxide production	ALBUM trial
HIF stabilizer • PHD inhibitor	Activation of HIF pathway	Phase 2 or phase 3 clinical trials of roxadustat, daprodustat, molidustat, vadadustat, and enarodustat

ALBUM, a study to evaluate ASP8232 as add-on therapy to angiotensin converting enzyme inhibitor (ACEi) or angiotensin receptor blocker (ARB) in reducing albuminuria in patients with type 2 diabetes and chronic kidney disease; ASK-1, apoptosis signal-regulating kinase 1; AYAME, a phase 3 study of bardoxolone methyl in patients with diabetic kidney disease; BEACON, bardoxolone methyl evaluation in patients with chronic kidney disease and type 2 diabetes; BEAM, trial to determine the effects of bardoxolone methyl on estimated glomerular filtration rate in patients with type 2 diabetes and chronic kidney disease; HIF, hypoxia-inducible factor; JNK, c-Jun amino terminal kinase; KEAP1, Kelch-like ECH-associated protein 1; MAPK, mitogen-activated protein kinase; Nrf2, nuclear factor erythroid 2-related factor 2; PHD, prolyl hydroxylase; TSUBAKI, the phase II study of bardoxolone methyl in patients with chronic kidney disease and type 2 diabetes; VAP-1, vascular adhesion protein-1.

have shown beneficial results in clinical trials (Table 2). Future studies are needed to accumulate more evidence of the renoprotective effects and safety of those agents in clinical applications.

Conflicts of interest

All authors have no conflicts of interest to declare.

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Authors' contributions

All of the authors contributed to the formation of overall concept. Tomoko Honda wrote the manuscript and Yosuke Hirakawa and Masaomi Nangaku edited the manuscript. All authors read and approved the final manuscript.

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