The mechanism of hyperoxia-induced neonatal renal injury and the possible protective effect of Resveratrol

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Abstract:
With recent advances in neonatal intensive care, preterm infants are surviving into adulthood. Nonetheless, epidemiological data on the health status of these preterm infants has begun to reveal a worrying theme; prematurity and the supplemental oxygen therapy these infants receive after birth appear to be risk factors for kidney disease in adulthood, affecting their quality-of-life. As the incidence of chronic kidney disease and the survival time of preterm infants both increase, the management of hyperoxia-induced renal disease is becoming increasingly relevant to neonatologists. The mechanism of this increased risk is currently unknown, but prematurity itself and the hyperoxia exposure after birth may predispose to disease by altering the normal trajectory of kidney maturation. This article reviews altered renal reactivity due to hyperoxia, the possible mechanisms of renal injury due to hyperoxia, and the role of resveratrol in renal injury.

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The mechanism of hyperoxia-induced neonatal renal injury and the possible protective effect of Resveratrol

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Abstract

With recent advances in neonatal intensive care, preterm infants are surviving into adulthood. Nonetheless, epidemiological data on the health status of these preterm infants has begun to reveal a worrying theme; prematurity and the supplemental oxygen therapy these infants receive after birth appear to be risk factors for kidney disease in adulthood, affecting their quality-of-life. As the incidence of chronic kidney disease and the survival time of preterm infants both increase, the management of hyperoxia-induced renal disease is becoming increasingly relevant to neonatologists. The mechanism of this increased risk is currently unknown, but prematurity itself and the hyperoxia exposure after birth may predispose to disease by altering the normal trajectory of kidney maturation. This article reviews altered renal reactivity due to hyperoxia, the possible mechanisms of renal injury due to hyperoxia, and the role of resveratrol in renal injury.
Keywords: resveratrol; hyperoxia; renal injury; CKD

Key points:

- Premature infants commonly receive supplementary oxygen.
- Hyperoxia can cause kidney damage via signal pathways.
- We should reduce the occurrence of late sequelae.

Introduction

Premature infants are often treated with supplemental oxygen after birth due to the immaturity of their cardiopulmonary function and antioxidant system at birth. In neonatal intensive care units, supraphysiological oxygen concentrations are often used in the treatment of critical neonatal resuscitation, pulmonary hypertension, and respiratory distress syndrome; however, although it saves the lives of premature infants, oxygen supplementation can have adverse effects. Numerous studies have shown that high concentrations of oxygen are toxic to the developing lungs and retina.\textsuperscript{1,2} At the same time, supraphysiological oxygen concentrations lead to apoptosis, degenerative responses, cell death, and reduced brain mass in the developing brain, with the induced cell death being diffuse and affecting the entire brain.\textsuperscript{3} In studies using mouse models, hyperoxia exposure has been found to cause damage to the white matter of developing brains, including changes in myelin ultrastructure and a reduction in myelin proteins.\textsuperscript{4-6} Moreover, hyperoxia is an important additional risk factor for increased neuronal death in infants with
intrapartum asphyxia and can worsen the outcome of neonatal asphyxia.\textsuperscript{7,8} A clinical study on hyperoxia and brain damage in preterm infants suggests that hyperoxia also increases the risk of cerebral palsy in preterm infants.\textsuperscript{9}

Injuries due to hyperoxia are easily associated with lung damage, but are often overlooked for certain non-target organs. As a metabolically-active organ, the kidney is usually susceptible to external stresses due to its abundant blood flow. Blood is ultrafiltrated through the various levels of the renal structures, such as the renal units, glomeruli, and capillary network, to maintain the stability of the internal environment and excrete exogenous substances, solutes, water, and various metabolic wastes. Yet, under pathophysiological conditions, when the above-listed processes are impeded, kidney function is affected and cell and tissue damage may occur.\textsuperscript{10} Research has produced conflicting results with respect to the role of oxygen in the kidney. Some studies have suggested that hyperoxia can activate appropriate signaling pathways to play a protective role in renal ischemia-reperfusion injury,\textsuperscript{11} and has a positive protective effect on peripheral vasoconstriction prior to infectious shock, maintaining blood pressure, stabilizing hemodynamics during vasodilatory shock, limiting the hyperinflammatory response in early infectious shock\textsuperscript{12} and hyperoxia during resuscitation from non-lethal shock.\textsuperscript{13} Studies of rabbits have shown that, after hyperoxia stimulation, their kidneys respond with an increase in tissue oxygenation.\textsuperscript{14} Human and other animal studies have also demonstrated that neonatal hyperoxia increases oxidative stress\textsuperscript{8} and leads to glomerular and tubular damage; these effects
manifest during the perinatal period as enlarged renal tubules, tubular necrosis, interstitial inflammation, and renal fibrosis.\textsuperscript{15,16}

In rats, renal genesis continues until approximately day 10, unlike in humans, where it is complete by week 36 of gestation. As such, exposure to hyperoxia immediately after birth in rats may have similar consequences to exposure to hyperoxia during the critical period of postnatal renal genesis in preterm infants.\textsuperscript{17} The early exposure to hyperoxia in rats is associated with a later onset of hypertension, vascular dysfunction, and reduced renal function in adulthood, suggesting that hyperoxia during nephrogenesis can have a detrimental effect on kidney development.\textsuperscript{18} Various studies have shown that prematurity and potential postnatal hyperoxia are associated with chronic kidney disease (CKD) in adulthood\textsuperscript{19-21} and can cause renal dysfunction,\textsuperscript{14,22-29} ultimately predisposing newborns to hypertension and CKD later in life.\textsuperscript{30-32} Some studies suggest that exposure to high oxygen levels during development may affect kidney function and increase susceptibility to kidney disease in adulthood, and correlate with age and sex.\textsuperscript{17}

Based on abundant data, in neonates, hyperoxia causes renal injury during resuscitation therapy performed on preterm infants and resveratrol may attenuate renal injury by reducing inflammation and oxidative stress. Theoretically, in a model of hyperoxia-induced lung injury, resveratrol supplementation has a protective effect on the kidneys and is a promising treatment; however, studies have not yet evaluated its efficacy. Therefore, the aim of this review was to describe the molecular mechanisms associated with renal injury after hyperoxia and the possible benefits of resveratrol on
the kidney by exerting inflammatory and oxidative stress regulation in a hyperoxia-induced lung injury model.

**The mechanism of hyperoxia-induced neonatal renal injury**

Compared to full-term infants, premature infants with immature kidney development have lower levels of enzymes and non-enzymatic antioxidants. Preterm birth itself may also bring about adverse renal outcomes. Premature infants show a more rapid progression of renal pathology, a higher risk of CKD in adulthood, and a predisposition to hypertension than full-term infants. Since preterm infants’ kidneys are developing, they are vulnerable to various factors, including hyperoxia. Hyperoxia exposure increases oxidative stress, causing impaired kidney development and kidney injury, but the mechanism behind hyperoxia-induced kidney injury is not fully understood.

Hyperoxia induces renal inflammation, as evidenced by the activation of renal nuclear factor-κB (NF-κB) and elevated levels of pro-inflammatory cytokine. In the immediate postnatal period, hyperoxia exposure may delay renal development. Hyperoxia exposure also increases tubular damage and total collagen content in the rat kidney in the first 3 weeks of life, which can lead to renal fibrosis. Moreover, the development of postnatal hyperoxic renal fibrosis is associated with the increased expression of connective tissue growth factors in renal tissue. Inflammation is a key mechanism that promotes interconnected fibrosis and cellular injury in the renal mesenchyme. Oxidative stress can establish a vicious cycle between inflammation and cytokines by activating NF-κB to promote the persistence of inflammatory-
oxidative stress and the further overproduction of multiple cytokines.\textsuperscript{37} In contrast, oxidative stress due to hyperoxia is a potent activator of NF-κB.\textsuperscript{38} Therefore, hyperoxia may promote the process of renal fibrosis by activating NF-κB.

A rodent model of hyperoxia-induced kidney injury demonstrated that hyperoxia induces restricted renal perfusion, glomerulomegaly, and higher tubular injury scores. Additionally, in rodents, exposure to hyperoxia during early nephrogenesis in the postnatal period was accompanied by a significant reduction in the expression of renal klotho.\textsuperscript{39} Hyperoxia-induced acute kidney injury (AKI) has also been studied in two phases, with the early phase associated with the inhibition of kidney growth and the activation of IL-6 and Smad2 signaling. During the second phase, catch-up growth as well as impaired glomerular and tubular function occurs, and the IL-6/Smad2 signaling axis mediates AKI and regeneration in a hyperoxic mouse model, increasing the risk of CKD in adulthood.\textsuperscript{40} Hyperoxia induces oxidative stress, and the association between oxidative stress and nephrotoxicity has been largely attributed to the production of reactive oxygen species (ROS); these can promote epithelial-to-mesenchymal transition (EMT) in the kidney via the transforming growth factor-β/smad (TGF-β/smad) pathway.\textsuperscript{41} Neonatal hyperoxia downregulates mitogen-activated protein kinases (MAPK) signaling by regulating cell cycle arrest, progenitor cell maintenance, and differentiation, and the MAPK signaling pathway plays a key role in nephrogenesis.\textsuperscript{42-44} Data from one study indicates that neonatal hyperoxia impairs proximal tubule development accompanied by alterations in MAPK/extracellular signal-regulated kinase signaling and downregulation of
hypoxia-inducible factor-1α (HIF-1α) and catalase (CAT). An additional study suggested that the mechanism of hyperoxic nephropathy may be related to impaired tight junction proteins in the kidney, and that neonatal hyperoxia extensively inhibits the expression of tight junction proteins during proximal tubule development, possibly by downregulating proximal tubule claudin-4 expression and perhaps mediated through IL-6 and TNF-α; however, the inhibition of expression mediated by IL-6 and TNF-α are only involved in hyperoxia-induced renal damage and are not dependent on their proinflammatory effects. Moreover, a mouse model study found no change in the number of kidney units in mice exposed to 65% oxygen and no significant long-term deleterious effects on glomerular structure. Conversely, early exposure to 80% oxygen resulted in hypertension, changes in vascular function, and reduced kidney unit function in adulthood. These manifestations may be related to the oxygen concentration, the type of mice used, and the environment, such that the exact oxygen concentration that cause kidney damage requires further investigation. Nonetheless, hyperoxia exposure significantly reduces HIF-1α expression while kidney development remains ongoing.

NF-κB and aquaporin-1 (AQP-1) are thought to be signal transduction pathways in hyperoxic lung injury; hyperoxia activates this pathway by increasing nuclear translocation and inhibiting AQP-1 expression. In contrast, in a neonatal rat model of acute hyperoxic lung injury, AQP-1 is abundantly expressed in proximal renal tubular cells and the expression of AQP-1 decreases with increasing renal tissue
injury, suggesting that aquaporins play an important role in the hyperoxic renal injury process.

Angiopoietins (Angpts) are widely expressed in the fetal kidney, play a key role in renal vascular maturation, and are natural ligands and receptors with Tie-2; studies have shown that renal vascular configuration is disrupted in mice defective in the Angpt-2 gene. Moreover, numerous studies of hyperoxia and lung injury suggest Angpts as a target for hyperoxia-induced lung injury. Therefore, hyperoxia may also cause renal injury by affecting Angpts. A study of the renal damage occurring in neonatal rats with oxygen-derived retinopathy suggests that renal damage in preterm infants may be the result of altered microangiogenesis caused by ROS and vascular endothelial growth factor-A (VEGF-A; Figure 1).

The possible protective effect of Resveratrol (RSV)

With medical technology advances and a marked increase in the proportion of the preterm population surviving into childhood and adulthood, there is a growing need to better understand the long-term effects of preterm birth and postnatal exposure to stimuli such as hyperoxia on the kidneys. The Global Burden of Disease Study highlights CKD as a growing public health problem, with an increasing contribution to global mortality. A study in the United States indicated that CKD is associated with severe morbidity mortality, affecting an estimated 14% of the US population. In recent years, kidney disease has gained increasing attention due to its rapidly increasing prevalence worldwide, as well as the lack of effective treatments. Despite intensive research into the complex pathogenesis of kidney
diseases such as AKI and CKD, the morbidity and mortality rates of AKI and CKD continue to rise. The period from 4 to 6 weeks after birth is extremely important for establishing the quality of the renal unit in preterm infants, and postnatal renal injury should be avoided as much as possible. Therefore, methodologies to mitigate and protect the developing kidney are a current global concern and difficult issue.

Several studies using animal models have shown that Mesenchymal stem cells (MSCs) are able to promote renal repair through a variety of mechanisms, and therapies based on regenerative medicine aspects such as MSCs have great potential for the treatment of renal diseases. In addition, low-intensity shockwave therapy can improve or even reverse the microvascular loss, ischemia, and inflammation exhibited by kidney disease, ultimately slowing the progression of CKD. In the meantime, gene therapy, CRISPR gene editing techniques, and fatty acid-derived specialized pro-catabolic mediators and other targeted therapies for inflammation have begun to emerge as potential treatments. However, based on traditional experience and their multi-targeting characteristics, natural products, such as as well as Ganoderma lucidum, should increasingly be considered an alternative source of treatment for kidney disease.

Resveratrol (RSV) has gained attention for its unique antioxidant, anti-inflammatory, anti-diabetic, hepatoprotective, neuroprotective and anti-cancer properties. As a natural phenolic compound, RSV has been shown to have good therapeutic effects on a variety of diseases. RSV attenuates oxidative stress through the adenylate-activated protein kinase (AMPK)/silencing information regulator 1
(SIRT1) non-dependent pathway and reduces endoplasmic reticulum stress by regulating angiogenesis and through the protein kinase R-like endoplasmic reticulum kinase (PERK) pathway, thereby reduce the damage of diabetic nephropathy (DN).\textsuperscript{66} Moreover, RSV is protective against DN by activating AMPK-SIRT1-peroxidase-proliferation-activated receptor α (PPARα) via the Adipose Receptor AdipoR1 and AdipoR2, thereby increasing circulating lipocalin levels and reducing inflammation, oxidative stress, apoptosis, and endothelial dysfunction.\textsuperscript{67} Furthermore, the expression of heat shock protein 70 (HSP70) may exert a beneficial effect on renal injury by inhibiting inflammation and reducing the expression of inflammatory factors.\textsuperscript{68} As such, RSV may improve renal function in uremic rats by increasing the expression of Hsp70; an additional report suggests that it exerts a renoprotective effect by regulating the expression of NF-κB pathway-related proteins.\textsuperscript{69} Furthermore, RSV has been reported to exert anti-cancer effects on human kidney cancer cells by inhibiting the expression of the VEGF gene.\textsuperscript{70} Since nuclear factor E2-related factor 2 (Nrf2) controls key cellular defense responses against oxidative stress, the Nrf2 signaling pathway plays a key role in defending against oxidative stress and inflammatory responses.\textsuperscript{71} As such, activation of Nrf2 is important for maintaining redox homeostasis in aging-related renal injury; as an Nrf2 agonist, RSV improves renal function, proteinuria, and pathological changes by reducing oxidative stress, an effect that is closely associated with mitochondrial dysfunction. Furthermore, RSV also increases SIRT1/AMPK signaling and reduces oxidative stress as well as mitochondrial dysfunction by regulating peroxisome proliferator-activated receptor
gamma coactivator 1α (pGC-1α) and estrogen-related receptor α (ERRα). Data also confirm that SIRT1 has a positive protective effect against kidney injury, heart failure, and lung injury, and that RSV can improve kidney function and histological abnormalities through SIRT1.

Many current studies suggest that oxidative stress can promote mitochondrial dysfunction and that mitochondrial biogenesis is induced during aging, but also plays an important role in oxidative stress. RSV regulates mitochondrial biogenesis by modulating the cellular signaling pathways that maintain a stable intracellular environment, upregulates α deacetylation of SIRT1 and PGC-1 both in vivo and in vitro, improves antioxidant capacity by activating SIRT1 and PGC-1α, and attenuates renal hypoxia, mitochondrial dysfunction, and renal tubular apoptosis in rats with DN by enhancing the SIRT1-PGC-1-HIF-1α signaling pathway. A vast body of evidence indicates that SIRT1 plays a major role in various renal diseases; it has been shown to alleviate DN at the cellular level by activating the AMPK/SIRT1 pathway and regulating angiogenesis, in addition to regulating autophagy and oxidative stress responses in DN. Current investigations have demonstrated a potential relationship between dysbiosis of the intestinal flora and disruption of intestinal barrier function with the onset of CKD, in addition to a significant correlation between intestinal flora and hyperoxia-induced bronchopulmonary dysplasia. Thus, hyperoxia may also contribute to kidney disease by destabilizing the intestinal flora, whereas RSV may exert its protective effect against kidney disease by maintaining the intestinal flora and gut in a stable and healthy state.
Moreover, in a CKD model, RSV attenuates glomerular damage in the remnant kidneys of nephrectomized rats, increases mitochondrial membrane potential, increases adenosine triphosphate (ATP), reduced ROS production, and improved mitochondrial function in vitro and in vivo.\textsuperscript{75}

As for the renoprotective effects of RSV, when applied prior to nephrectomy, RSV is effective in alleviating the reduction in glomerular count caused by renal heat ischaemia.\textsuperscript{87} The relationship between oxidative stress and nephrotoxicity has been attributed to the production of ROS, which promotes the stimulation of the pro-inflammatory mediator TGF-β1, activates the Smad signaling pathway, and facilitates the initiation process of EMT.\textsuperscript{20,41,88} RSV effectively inhibits gentamicin-induced EMT by suppressing oxidative stress and possibly participating in the TGF-β/Smad signaling pathway.\textsuperscript{41} RSV also protects the kidney from oxidative stress in a CAT-dependent and glutathione peroxidase (GPX) non-dependent manner, and contrary to previous studies, one study suggests that high expression of SIRT1 may increase nephrotoxicity.\textsuperscript{10} Moreover, RSV significantly reduces serum creatinine (Scr), blood urea nitrogen and renal index levels, inhibits the expression of Fas-ligand (Fas-L) and TNF-α, and regulates the expression of downstream signaling effectors Caspase-8, BH3 interacting domain (Bid), Bcl-2 associated protein X (Bax) and B cell lymphoma-2 (Bcl-2), thereby achieving renoprotective effects.\textsuperscript{89} In addition, RSV protects the kidney from lipopolysaccharide (LPS) damage by modulating the immune response through a variety of pathways, such as reducing cytokine concentrations, decreasing vascular permeability, inhibiting macrophage activation,
and inducing macrophage apoptosis.\textsuperscript{90} RSV is suggested to protect against aging kidneys by reducing oxidative stress, inflammation, and fibrosis through inhibition of angiotensin-converting enzyme II and activation of angiotensin-converting enzyme.\textsuperscript{91} Furthermore, in mice, RSV ameliorates contrast-induced nephropathy through the activation of SIRT1-PGC-1α-Forkhead box protein O1(FOXO1) signaling.\textsuperscript{92} Mitochondrial quality control (QC)-associated proteins (Drp1, PINK1, Parkin, BNIP3 and PGC1) are reported to be involved in renal injury and may be involved in the nephroprotective effects of RSV.\textsuperscript{93} Moreover, renal fibrosis may be the result of activation of the TGF-β signaling pathway; RSV can target the TGF-β signaling pathway, thus effectively improving renal fibrosis (Figure 2).\textsuperscript{94}

**Summary**

Overall, in both human and animal studies, early exposure to hyperoxia has been shown to increase oxidative stress and cause renal injury via different signaling pathways. Meanwhile, RSV can exert protective effects against other models of renal disease via the same pathways as hyperoxia-induced renal injury, such as DN and AKI by inhibiting ROS and HIF-α to attenuate mitochondrial dysfunction. Therefore, it is reasonable to speculate that RSV may exert protective effects against post-hyperoxic kidney injury through its common pathway. RSV may become an effective drug for the treatment of post-hyperoxic kidney injury; however, its specific signaling pathway is not yet supported by strong evidence, and further studies are needed.
Authors’ contributions Yunchuan Shen wrote the manuscript. Yuan Yuan provides support in investigation and data collation. Wenbin Dong audited the manuscript. All authors contributed to the article and approved the submitted version.

Conflict of Interest None declared.

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**Figure legends**

**Figure 1** The kidney can be stimulated by hyperoxia through different signaling pathways and different mechanisms to promote renal injury. (+): stimulation;(-) inhibition.

**Figure 2** Role of resveratrol in kidney injury protection. This schema summarizes how resveratrol exerts its nephroprotective effects through different signaling pathways to alleviate kidney damage due to various causes. Different colors represent different signaling pathways.(+): stimulation;(-) inhibition.