Chronic Hypoxia and Tubulointerstitial Injury: A Final Common Pathway to End-Stage Renal Failure

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Recent studies emphasize the role of chronic hypoxia in the tubulointerstitium as a final common pathway to end-stage renal failure. When advanced, tubulointerstitial damage is associated with the loss of peritubular capillaries. Associated interstitial fibrosis impairs oxygen diffusion and supply to tubular and interstitial cells. Hypoxia of tubular cells leads to apoptosis or epithelial-mesenchymal transdifferentiation. This in turn exacerbates fibrosis of the kidney and subsequent chronic hypoxia, setting in train a vicious cycle whose end point is ESRD. A number of mechanisms that induce tubulointerstitial hypoxia at an early stage have been identified. Glomerular injury and vasoconstriction of efferent arterioles as a result of imbalances in vasoactive substances decrease postglomerular peritubular capillary blood flow. Angiotensin II not only constricts efferent arterioles but, via its induction of oxidative stress, also hampers the efficient utilization of oxygen in tubular cells. Relative hypoxia in the kidney also results from increased metabolic demand in tubular cells. Furthermore, renal anemia hinders oxygen delivery. These factors can affect the kidney before the appearance of significant pathologic changes in the vasculature and predispose the kidney to tubulointerstitial injury. Therapeutic approaches that target the chronic hypoxia should prove effective against a broad range of renal diseases. Current modalities include the improvement of anemia with erythropoietin, the preservation of peritubular capillary blood flow by blockade of the renin-angiotensin system, and the use of antioxidants. Recent studies have elucidated the mechanism of hypoxia-induced transcription, namely that prolyl hydroxylase regulates hypoxia-inducible factor. This has given hope for the development of novel therapeutic approaches against this final common pathway.


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together again at the vascular pole to form the efferent arterioles. Efferent arterioles enter the peritubular capillary plexus, which surrounds tubules and offers oxygen and nutrients to tubular and interstitial cells (Figure 1).

Although blood flow to the kidney is high, accounting for 20% of cardiac output, the presence of oxygen shunt diffusion between arterial and venous vessels that run in close parallel contact means that renal tissue oxygen tensions are in fact comparatively low (19,20). Oxygen tension in the renal medulla, for example, does not rise above 10 mmHg. That in the renal cortex is more variable, however, with an average pO2 of approximately 30 mmHg, but decreases dramatically in accordance with changes in renal perfusion. As a consequence, the kidney is somewhat sensitive to changes in oxygen delivery. Although this sensitivity has the merit of facilitating the kidneys in their adjustment of erythropoietin (EPO) production to changes in oxygen supply, it also renders them prone to hypoxic injury.

The chronic hypoxia hypothesis, proposed by Fine et al. (21), emphasizes chronic ischemic damage in the tubulointerstitium as a final common pathway in end-stage kidney injury. Since its introduction, this fascinating hypothesis has been investigated intensively and subsequently validated by Eckardt, Johnson, and many other investigators (22–24).

Chronic Hypoxia in the Kidney Is Multifactorial

Loss of Peritubular Capillaries and Fibrosis in Chronic Renal Disease

Chronic ischemia in the tubulointerstitium occurs via several mechanisms acting in concert. Histologic studies of human kidneys and animal models have shown that extensive tubulointerstitial injury is associated with damage to renal arterioles and arteries as well as with distortion and loss of peritubular capillaries (25–29). It therefore is of little wonder that fibrotic kidneys with advanced renal disease are devoid of peritubular capillary blood supply and oxygenation to the corresponding region (Figure 2A).

Even when the peritubular capillaries are essentially intact, however, interstitial fibrosis still impairs tubular oxygen supply. This is because the extended distance between the capillaries and tubular cells reduces the efficiency of oxygen diffusion (Figure 2B). In this regard, it is notable that hypoxia per se is a profibrogenic stimulus for tubular cells, interstitial fibro-
blasts, and renal microvascular endothelial cells. Tubular cells under hypoxic conditions undergo epithelial-mesenchymal transdifferentiation to become myofibroblasts (30). Hypoxia can also activate fibroblasts and change the extracellular matrix metabolism of resident renal cells (31,32). A fibrogenic response leads in turn to the obliteration of peritubular capillaries. Furthermore, renal tubular cells that are subjected to severe or prolonged hypoxia develop in their mitochondria functional deficits that lead to persistent energy deficits, subsequently causing them to undergo apoptosis (33). Together, chronic hypoxia in this compartment can lead to transdifferentiation or apoptosis (or both) of tubular cells, activation of resident fibroblasts, and further obliteration and loss of peritubular capillaries with progression of fibrosis. These changes may combine to institute a vicious cycle of regional hypoxia and progressive kidney failure in the late stages of disease.

**Glomerular Damage and Hypoxia of the Tubulointerstitium**

Hypoxia also plays a pathogenic role in the relatively early stages of kidney disease, well before the development of structural tubulointerstitial injury. Peritubular capillaries occur downstream of the glomerular efferent arterioles. Impairment of the “parent” glomerular capillary bed, as occurs in glomerulosclerosis, for example, thus automatically results in a decrease in peritubular perfusion and tubular oxygen supply (Figure 2C). In a model of accelerated glomerulosclerosis induced by repeated injection of anti-Thy1 antibody in uninephrectomized rats, we observed a decrease in blood flow in peritubular capillaries using intravital microscopy and physiologic lectin perfusion (34). Stagnation of peritubular capillary blood flow was associated with hypoxia in the corresponding tubulointerstitium, and both preceded the development of histologic tubulointerstitial injury and peritubular capillary loss.

**Hemodynamic Maladjustment in the Tubulointerstitium: Imbalance of Vasoactive Substances**

Even in the presence of structurally intact glomeruli, imbalances in vasoactive substances and associated intrarenal vasoconstriction can cause chronic hypoxia in the kidney in the early stage of kidney disease, before the development of histologic changes in the tubulointerstitium (Figure 2D). Futrakul et al. (35) performed intrarenal hemodynamic studies in patients with severe glomerulonephritis using radioisotope techniques and showed that elevated efferent arteriolar resistance and decreased peritubular capillary flow were associated with reversible renal functional impairment. This reversible change in peritubular capillary flow may have reflected an improvement in the imbalance of vasoactive substances in the kidney. They recently extended these observations to report a correlation between a decrease in peritubular capillary flow and tubular dysfunction in patients with type 2 diabetes and normoalbuminuria (36). These results support the concept that chronic hypoxia may/can induce tubulointerstitial injury, which eventually leads to ESRD in patients with a variety of kidney diseases.

Among various vasoactive substances, local activation of RAS is especially important because it can lead to constriction of efferent arterioles, hypoperfusion of postglomerular peritubular capillaries, and subsequent hypoxia of the tubulointerstitium in the downstream compartment. To clarify the mechanism of these effects, we used a remnant kidney model in rats induced by ligation of renal artery branches, in which RAS is markedly activated. Our computer-assisted morphologic analysis demonstrated narrowing and distortion of peritubular capillaries with decreased blood flow and hypoxia in a very early phase in this model, before the development of structural kidney damage (37). In addition, angiotensin II damages endothelial cells directly: Administration of angiotensin II to rats causes the loss of peritubular capillaries, an effect that is ameliorated by receptor blockade (38,39). A second important mechanism of angiotensin II–induced ischemia is inefficient cellular respiration and hypoxia via oxidative stress, which is detailed below. Thus, angiotensin II induces tubulointerstitial hypoxia via both hemodynamic and nonhemodynamic mechanisms. Intrarenal vasoconstriction may also occur secondary to increased local endothelin or a local loss of vasodilating nitric oxide (NO).

**Role of Anemia in Hypoxia of the Kidney**

The amount of O2 delivered, either to the whole body or to specific organs, is the product of blood flow and arterial O2 content. Under most circumstances, oxygen delivery (DO2) is determined using the equation DO2 = CO × (%Sat × 1.39 × [Hb]), where CO is cardiac output in liters per minute, %Sat is percentage of hemoglobin O2 saturation, [Hb] is hemoglobin concentration in grams per liter, and 1.39 is the hemoglobin binding constant. From the equation, anemia in kidney disease may accelerate the decline in renal function by inducing tubulointerstitial hypoxia (Figure 2G). The important role of anemia is emphasized by the fact that anemia is observed at a relatively early stage of renal dysfunction. Both the Third National Health and Nutrition Examination Survey and the National Kidney Foundation Kidney Early Evaluation Program showed that the risk for anemia significantly increases when GFR falls below 60 ml/min per 1.73 m2 (40,41). Studies that have confirmed anemia as an independent risk factor for ESRD include a retrospective multivariate logistic analysis of 71,802 subjects that was performed by Iseki et al. (42) and an analysis of the data of the Reduction of Endpoints in NIDDM with the Angiotensin II Antagonist Losartan study of patients with type 2 diabetic nephropathy (43). The average increase in adjusted relative risk in the latter study was 11% for each 1-g/dl decrease in hemoglobin concentration.

**Oxidative Stress and Inefficient Cellular Respiration**

Chronic kidney disease is associated with oxidative stress. Angiotensin II, which is often upregulated in renal diseases, also promotes renal oxidative stress by stimulating NADPH oxidase. Furthermore, renal anemia contributes to oxidative stress as erythrocytes represent a major antioxidant component of the blood.

Superoxide leads to decreased NO bioavailability through ONOO– formation. Adler et al. (44) showed that, because NO is a suppressor of mitochondrial respiration, depletion of NO by oxidative stress may stimulate mitochondrial respiration and...
uncouple it from chemical energy consumption, resulting in tissue hypoxia (Figure 2E).

Kidneys of the spontaneously hypertensive rat (SHR), which characteristically undergo oxidative stress, revealed enhanced oxygen usage relative to tubular sodium transport and lower intrarenal \( \text{PO}_2 \) (45). Amelioration of oxidative stress improved renal oxygenation in a model of diabetic nephropathy (46) and in the angiotensin II continuous infusion model (47). The same oxidative stress–related mechanism may cause tubulointerstitial hypoxia in the aging kidney (48). It is likely that the renal hypoxia in these models results from a decrease in NO bioavailability and subsequent uncoupling of mitochondrial respiration as a result of oxidative stress.

Relative Hypoxia as a Result of Increased Metabolic Demand

When metabolic demand is increased, cells may suffer from relative hypoxia even under the maintenance of otherwise normal blood flow. Studies that have used the blood oxygen level–dependent (BOLD)–magnetic resonance imaging (MRI) technique (see Detection of Hypoxia in the Kidney section) have demonstrated that streptozotocin-induced diabetic kidneys suffer from tissue hypoxia at an early stage, before the development of structural changes (49). A possible explanation is that the hyperfiltration that occurs early in diabetic nephropathy leads to the increased delivery of sodium to tubular cells, imposing an excessive tubular sodium reabsorption workload relative to oxygen supply and subsequently resulting in tubular hypoxia (Figure 2F). Whether proteinuria causes functional hypoxia as a result of increased metabolic demand for reabsorption is an important question for future study.

Detection of Hypoxia in the Kidney

Despite an ever-increasing need for methods to identify and quantify hypoxic cells in vivo, suitable tools for detecting low oxygenation within tissues remain in short supply. Among those with potential diagnostic and research use are chemical tools such as pimonidazole, which is reduced under conditions of low oxygen availability. Visualization of this reaction allows us to detect hypoxic cells. These chemical methods are subject to a number of limitations, however: Their sensitivity is relatively low, detecting hypoxic cells at oxygen levels of <10 mmHg only, and they are not quantitative. Moreover, the hypoxia probe is metabolized and bound to cells over a 1- to 3-h period, requiring the assumption that oxygen content as well as delivery of the chemical compound, in terms of blood flow to the tissue, remain constant over the observation period. An additional limitation is that ischemia might impair the delivery of the compound to hypoxic tissues.

Polarographic oxygen sensors serve as true oxygen monitors, but the method is invasive and functional in only a limited range of tissues. In addition, because their signal is proportional to the measured quantity, they can become noisy and inaccurate, especially at low oxygen levels over relatively large tissue volumes.

To overcome these problems, Tanaka from our group recently established a novel transgenic rat (50). These animals, which were highlighted in a recent issue of the JASN (51), express luciferase tagged with FLAG under a promoter composed of a tandem repeat of hypoxia-inducible factor (HIF) binding sites, providing a wide dynamic detection range of quantitative oxygen concentration with resolution down to the individual cell level. These animals enabled us to demonstrate different patterns of hypoxia at the early stage in various kidney disease models. An impressive regional correlation was noted between areas of hypoxia and areas of macrophage accumulation, apoptosis, and cell proliferation.

With regard to future clinical applications, BOLD-MRI is a promising tool for the estimation of tissue oxygenation in vivo. Whereas oxyhemoglobin is diamagnetic, deoxyhemoglobin is paramagnetic. Thus, when red blood cells that contain deoxyhemoglobin are placed in the magnetic field of an MRI, they cause field distortion, which appears as BOLD contrast in the resulting images. Limitations at this time include difficulty in obtaining reproducible and reliable information in this mobile organ, i.e., the kidney.

Therapeutic Approaches to Chronic Hypoxia

Because chronic hypoxia in the tubulointerstitium is a final common pathway to ESRD, therapeutic approaches that target the chronic hypoxia should prove effective against a broad range of renal diseases. Potential treatment modalities that target chronic hypoxia in the kidney are summarized in Table 1. Details of each are discussed in the following sections.

Table 1. Treatment modalities that target chronic hypoxia in the kidney

<table>
<thead>
<tr>
<th>Treatment Targeting Hypoxic Tubulointerstitial Damage: EPO</th>
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<td>Improvement of anemia by EPO</td>
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<tr>
<td>Preservation of peritubular capillary blood flow by blockade of the renin-angiotensin system</td>
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<td>Protection of the vascular endothelium</td>
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<td>VEGF</td>
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<td>Antioxidants to improve the efficiency of cellular respiration</td>
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<td>HIF-based therapy</td>
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<td>prolyl hydroxylase inhibitors</td>
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<td>gene transfer of constitutively active HIF</td>
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*EPO, erythropoietin; VEGF, vascular endothelial growth factor; HIF, hypoxia-inducible factor.
hemoglobin decreased below 9 g/dl. The results clearly showed that early initiation of EPO in predialysis patients with anemia significantly slows the progression of renal disease. However, some other trials, including the much larger Cardiovascular Risk Reduction by Early Anemia Treatment with Epotin β trial, could not confirm beneficial effects of intensive treatment with EPO, and renoprotective effects of EPO requires further investigation.

**Blockade of RAS to Ameliorate Tubulointerstitial Hypoxia**

Norman et al. (53) were the first to show that blockade of RAS preserved peritubular capillary perfusion and tissue oxygenation in healthy anesthetized rats. In a remnant kidney model, we demonstrated that treatment with the angiotensin receptor blocker olmesartan restored blood flow in peritubular capillaries and improved oxygenation of the kidney (37). Although these improvements in kidney oxygenation by RAS inhibition are multifactorial, one important mechanism is the dilution of efferent glomerular arterioles and consequent increase in blood supply to the downstream tubulointerstitium. Inhibitors of RAS also serve as antioxidants and should ameliorate uncoupling of mitochondrial respiration, leading to more efficient use of oxygen. Supporting the latter mechanism, administration of an angiotensin receptor blocker corrected the reduced pO2 in the cortices of the SHR and reversed the inefficient use of O2 for Na+ transport (54).

**Protection of the Tubulointerstitial Vasculature**

Protection of the tubulointerstitial vasculature theoretically should preserve blood supply and guarantee oxygenation to the corresponding compartment. Physiologically, endothelial cells are covered with a layer of heparan sulfate proteoglycans, which are crucial to the anticoagulant and anti-inflammatory properties of the endothelium. Endothelial cell injury is associated with the loss of these proteoglycans on the cell surface and thrombus formation, followed by subsequent ischemic tubulointerstitial damage. On this basis, we hypothesized that administration of dextran sulfate may protect the kidney from endothelial damage by re-establishing the intact endothelial surface. To investigate this, we used a model of thrombotic microangiopathy induced by renal artery perfusion of an antiglomerular endothelial antibody. Results showed that the administration of dextran sulfate protected the kidney against endothelial damage, probably by acting as a “repair coat” (55) to re-establish the intact anticoagulant and anti-inflammatory surface of the injured endothelium.

Kang et al. (56) treated rats with remnant kidneys with vascular endothelial growth factor (VEGF). This treatment improved renal function and lowered mortality rates compared with the vehicle control, and histology confirmed an increase in peritubular capillary endothelial cell proliferation and a decrease in peritubular capillary rarefaction. These results showed that treatment with VEGF protected the kidney by both the preservation of the capillary endothelium and the partial reversal of the impaired angiogenesis.

**HIF as a Target for Drug Development**

Although VEGF is a promising therapeutic modality, a potential pitfall of the induction of vessels by overexpression of a single gene such as VEGF is that the resulting vessels may be leaky, immature, or irregular. This is because the formation of a functionally intact microvasculature requires the coordinated activation of various genes. Rather, a more promising approach to protecting tissues against hypoxia is the activation of a “master gene” switch that results in a broad and coordinated downstream reaction.

At the center of the cellular response to hypoxia is HIF (57,58). HIF is composed of two subunits, an oxygen-sensitive HIF-α subunit and a constitutively expressed HIF-β subunit (also known as aryl hydrocarbon receptor nuclear translocator [ARNT]). The first isoform of HIF-α, HIF-1α, was originally identified and cloned as a high-affinity DNA binding protein localized to the 3’ hypoxia-responsive element of the EPO gene (59,60). Both HIF-1α and HIF-1β are members of the basic helix-loophelix PER/ARNT/SIM (HLH-PAS) family of transcription factors. HIF binds to the hypoxia-responsive element in the cis-regulatory regions of its target genes and transcriptionally activates various genes encoding proteins that mediate adaptive responses to reduced oxygen availability.

Under normoxic conditions, two conserved proline residues within the central oxygen-dependent degradation domains of the HIF proteins are hydroxylated by the protein products prolyl hydroxylase domain containing (PHD) (61). This promotes binding of the von Hippel Lindau tumor suppressor protein, part of a ubiquitin ligase complex, resulting in polyubiquitlation and rapid degradation. Similarly, a conserved asparagine residue in the carboxyl-terminal transactivation domain of the HIF proteins is hydroxylated in normoxia by factor inhibiting HIF (FIH), preventing recruitment of the p300/CREB-binding protein transcriptional co-activators and thus leading to transcriptional repression. Under hypoxia, oxygen is lacking as an essential substrate for the hydroxylation reaction, and the unmodified HIF proteins avoid degradation but rather heterodimerize with HIF-β and upregulate the transcription of target genes. The biologic significance of HIF in the kidney under physiologic and pathologic conditions was demonstrated recently by Manoatham et al. from our group, who used an in vivo gene transfer of DNA expressing negative dominant HIF and constitutively active fusion protein of HIF (62).

Owing to its ability to induce the expression of a variety of oxygen-regulated and renoprotective genes in a coordinated and physiologic manner, stimulation of HIF-1 signaling may be more effective in ischemic states. For emphasizing the efficacy of this “master gene” switch, transgenic mice expressing constitutively active HIF-1α in the epidermis displayed an increase in dermal capillaries with a 13-fold elevation of VEGF (63). Despite a marked induction of hypervascularity, HIF-1α did not induce edema, inflammation, or vascular leakage, phenotypes that develop in transgenic mice that overexpress VEGF in skin.

A recently discovered isoform of HIF-1α, HIF-2α, has been shown to possess both structural and functional similarity to HIF-1α. HIF-1α and HIF-1β are expressed in most cell types,
whereas HIF-2α shows a more restricted pattern of expression (57,64). To study the expression of HIF-1α and HIF-2α in the kidney, Eckardt’s group used high-amplification immunohistochemical analyses (65,66) and showed that HIF-2α was induced by hypoxia in peritubular endothelial cells and fibroblasts as well as glomerular endothelial cells, whereas HIF-1α was localized predominantly in the tubular cells (65). These results are consistent with those of studies that have used a surrogate marker for HIF-2α in genetically engineered mice (67). In these mice, disruption of the murine HIF-2α gene was accomplished by homologous recombination in embryonic stem cells using a targeting plasmid in which a modified form of β-galactosidase (β-gal) was substituted for exon 2 of the HIF-2α gene. Activity staining for nuclear-localized β-gal revealed strong expression predominantly in vascular endothelial cells but also in the renal interstitial cell compartment, whereas β-gal staining was not evident in renal tubular cells.

Upregulation of the two HIF-α isoforms in the kidney by hypoxia was demonstrated in models of segmental renal infarction and radiocontrast nephropathy (68,69). Although cell-type specificity of HIF isoforms in these models was consistent with previous findings, temporal and spatial profiles of HIF activation were relatively complex, suggesting an important but complicated role of HIF in tissue preservation as a response to regional renal hypoxia. Our recent in vitro experiments showed that HIF-1α in tubular epithelial cells promotes proliferation of endothelial cells and that HIF-2α that is overexpressed in renal endothelial cells mediates migration and network formation; these results suggest a specific role of each isoform in certain cell types (70), although a clear differentiation of their roles independent of localization remains controversial.

Prolyl Hydroxylase

Three HIF prolyl hydroxylases with the potential to catalyze this reaction have been identified, and these proteins, termed PHD1, PHD2, and PHD3, seem to have arisen by gene duplication. The contribution of each to the physiologic regulation of HIF remains uncertain. These respective isoforms each have unique but overlapping patterns of tissue expression. Recent experiments using suppression by small interference RNA showed that each contributes in a nonredundant manner to the regulation of both HIF-1α and HIF-2α subunits and that the contribution of each PHD is strongly dependent on the abundance of the enzyme (71). In most cells, PHD2 has the most dominant effect because it is substantially the most abundant. Whereas both PHD2 and PHD3 proteins are induced by hypoxia, induction of PHD3 is particularly striking in certain cells, and under these conditions, the contribution of PHD3 is greater than that of PHD2. PHD3 seems to contribute more substantially to the regulation of HIF-2α.

Prolyl hydroxylase inhibitors have been the focus of recent studies on novel strategies to stabilize HIF. More than half a century ago, oral administration of cobaltous chloride was used to treat anemia associated with chronic renal disease (72). Cobalt therapy led to a significant erythropoietic response in association with improved appetite and greater tolerance for medications that are necessary to correct electrolyte abnormal-

### Conclusion

Chronic hypoxia is the final common pathway to end-stage renal failure. Ischemia of the kidney is induced by the loss of peritubular capillaries in the tubulointerstitium in the late stage of renal disease. Accumulating evidence also suggests a crucial role for hypoxia in the tubulointerstitium before structural microvasculature damage in the corresponding region, emphasizing the pathogenic role of this condition from an early stage of kidney disease. Given this background, therapeutic approaches against this final common pathway should be effective in a broad range of renal diseases. Presently, administration of EPO to correct anemia and blockade of RAS to preserve peritubular capillary flow and reduce oxidative stress are key to the improvement of kidney oxygenation. In the future, the HIF transcription factor at the center of many cellular hypoxic response pathways will be an attractive target for therapeutic manipulation.

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