Chronic Renal Ischemia in Humans: Can Cell Therapy Repair the Kidney in Occlusive Renovascular Disease?

Occlusive renovascular disease caused by atherosclerotic renal artery stenosis (ARAS) elicits complex biological responses that eventually lead to loss of kidney function. Recent studies indicate a complex interplay of oxidative stress, endothelial dysfunction, and activation of fibrogenic and inflammatory cytokines as a result of atherosclerosis, hypoxia, and renal hypoperfusion in this disorder. Human studies emphasize the limits of the kidney adaptation to reduced blood flow, eventually leading to renal hypoxia with activation of inflammatory and fibrogenic pathways. Several randomized prospective clinical trials show that stent revascularization alone in patients with atherosclerotic renal artery stenosis provides little additional benefit to medical therapy once these processes have developed and solidified. Experimental data now support developing adjunctive cell-based measures to support angiogenesis and anti-inflammatory renal repair mechanisms. These data encourage the study of endothelial progenitor cells and/or mesenchymal stem/stromal cells for the repair of damaged kidney tissue.

Atherosclerotic renal artery stenosis (ARAS) is common and produces gradual vascular occlusion of the kidney. Classically, ARAS is recognized when it produces renovascular hypertension, sometimes accelerating cardiovascular (CV) disease and impairing kidney function with substantial morbidity and mortality (54). Some degree of ARAS is common in older individuals and may reflect to a spectrum of changes ranging from minor narrowing of the renal arteries through focal hemodynamic stenosis reducing perfusion pressure, eventually leading to renal ischemia and kidney atrophy, to complete occlusion of renal blood flow (RBF) to one or both kidneys. The pathophysiological manifestations of ARAS are complex and change with disease progression (70). Landmark studies emphasize the transition between hemodynamic changes that require >60–80% vessel occlusion (18) and progressive stimulation of pressor mechanisms including activation of the renin-angiotensin-aldosterone system, sympato-adrenergic pathways, oxidative stress, and impaired vasodilatory responses that affect both renal and systemic microcirculations (5, 47–49). More recent studies highlight the activation of inflammatory pathways leading to transforming growth factor beta (TGF-β), macrophage accumulation, and eventual tissue fibrosis in experimental and human ARAS (33).

Hence, ARAS has especially complex manifestations in the kidney that often render simple restoration of blood flow insufficient to recover renal function (17, 78). This review will highlight recent clinical and experimental studies that suggest a role for cell-based therapy using autologous mesenchymal stromal/stem cells to repair kidney microvascular and interstitial structure and function.

Pathophysiology of Renovascular Disease

Renal Perfusion and Oxygenation in ARAS

The kidney normally is highly perfused, exhibits the highest rate of blood flow per tissue weight, and has the smallest arteriovenous differences in oxygen saturation of any organ, consistent with its primary function for blood filtration and limited overall net oxygen consumption (28, 59). As a result, the kidney is abundantly oxygenated compared with other organs. A striking feature, however, is the non-uniformity of tissue oxygenation within the kidney. Under normal conditions, a gradient of oxygenation develops within the renal parenchyma from a highly perfused cortex falling to much lower levels in the deep medulla, resulting from differences in both blood supply and oxygen consumption between the renal cortex and
medulla (3, 4). Portions of the medulla thus are functionally hypoxic under normal conditions and considered particularly susceptible to ischemic and/or other forms of acute kidney injury (4, 39, 45). Remarkably, normal kidneys tolerate these gradients without tissue injury under a wide range of physiological conditions (29, 77).

The kidney, or at least the cortical circulation, has a substantial “adaptive” capacity by which a reduction in renal perfusion and blood flow does not necessarily lead to local tissue hypoxia. The surplus of oxygenated blood combined with decreased single-kidney GFR and tubular reabsorption of sodium tends to preserve corticomedullary oxygenation (27, 43). However, at some point, severe ARAS reduces perfusion below the levels needed for oxygen delivery and leads to pathological renal ischemia.

When Does Reduced Blood Flow Magnify Tissue Hypoxia in ARAS?

Initially, the reduction of renal perfusion activates the renin-angiotensin-aldosterone (RAAS) and adrenergic stimuli, which themselves can generate reactive oxygen species and increase systemic oxidative stress (48). As vascular occlusion progresses with more severe and sustained stenosis, cortical hypoxia becomes prominent and leads to more oxidative stress, triggering inflammation and fibrosis pathways (10). These events ultimately lead to loss of kidney function, which may/may not be reversible after restoration of blood flow with revascularization (68).

Recent studies using oxygen-sensing electrodes in the pig during acutely produced renovascular occlusion demonstrate that regional renal tissue oxygenation declines (medullary more than cortical) (81). Renal cortical Po2 of chronically clipped kidneys is lower than contralateral kidneys in early Goldblatt hypertensive rats, supporting the existence of regional tissue hypoxia (61). These observations are consistent with imaging studies in pigs employing blood oxygen level-dependent (BOLD) magnetic resonance imaging (MRI) based on the local effects of tissue deoxyhemoglobin. BOLD MRI provides estimates of in vivo tissue oxygenation in humans non-invasively in real time by determining local levels of deoxyhemoglobin within the kidney (62, 64, 73). Hypoxia levels as assessed by BOLD MRI within the cortex and the medulla increase during gradual renal arterial occlusion (indirectly implying a rise in deoxyhemoglobin and, therefore, a decrease in renal oxygenation) (42). Furthermore, BOLD MR studies in kidneys of patients with ARAS under controlled conditions of sodium intake and drug therapy confirm they have higher overall hypoxia and reduced kidney functions compared with those with essential hypertension (67). Another BOLD MRI study included three groups of patients: essential hypertension, “moderate” ARAS, and “severe” ARAS and loss of functional renal tissue (32). Cortical hypoxia levels were higher in severe the ARAS group, showing the limits of kidney adaptation. Analysis of axial BOLD imaging using a “fractional hypoxia” method confirms that severe renovascular occlusion does indeed produce both cortical and overall tissue hypoxia (34, 67) that can revert to normal levels after restoration of vessel patency and increased renal blood flow (68) (FIGURE 1). These observations confirm that viable human kidneys may show regional ischemic changes that are associated with worsening tissue hypoxia.

The Role of Hypoxia in Renal Inflammation and Tubulointerstitial Fibrosis

Renal hypoxia has been implicated in the pathogenesis of acute kidney injury and in the progression of chronic renal disease (30). In vivo studies show that hypoxia alone is capable of initiating an immune response, macrophage accumulation, within the kidney (31). A recent study showed that renal tissue hypoxia induced by administration of a mitochondrial uncoupler, 2,4-dinitrophenol, by gavage to rats for a 30-day period increased renal oxygen consumption and led to renal tissue hypoxia in the absence of hypertension, hyperglycemia, and oxidative stress or even systemic hypoxemia. This injury leads to nephropathy characterized by proteinuria, increased renal vimentin expression, which is a marker of tubular damage, and infiltration of inflammatory cells (31). These data are supported by the presence of hypoxia as assessed by Pimonidazole immunohistochemistry in the early phases of the remnant kidney model of chronic kidney disease (51). Similarly, in addition to its hemodynamic effects, chronic stimulation of the RAAS contributes to the development of renal damage by enhancing expression of profibrotic cytokines (such as TGF-β) and growth factors, thus promoting tubulointerstitial fibrosis (79). Over time, these effects combine to mediate a transition from a primarily “hemodynamic” disorder to an “inflammatory” and fibrotic disorder (FIGURE 2). Hypoxia also stimulates oxidative stress through activation of superoxide production, xanthine oxidase, and NADPH oxidase (1), which likely also drive development of fibrosis (11, 30). Oxidative stress in renovascular disease has been defined by increased plasma isoprostanes and thiobarbituric acid reactive substances (TBARS), and reduced antioxidant enzymes such as glutathione peroxidise, superoxide dismutase, and catalase in porcine

176 PHYSIOLOGY • Volume 30 • May 2015 • www.physiologyonline.org
RVD uncomplicated by hypercholesterolemia (48). Renal biopsies of patients with ARAS show increased tubulointerstitial fibrosis and renal atrophy compared with normal kidneys (33). Moreover, chronic hypoxia can lead to epithelial cell apoptosis, since it activates inflammatory and profibrotic pathways and contributes to the pathogenesis of acute kidney injury (52, 56) that likely contributes to development of chronic kidney disease (55, 56). Clinical studies also have shown elevated renal vein levels of neutrophil gelatinase-associated lipocalin (NGAL) from the post-stenotic kidney of ARAS patients and metabolomic profiles consistent with CKD (22, 65, 68), as well as release of inflammatory markers (e.g., TNF-α and MCP-1) (68). Chronic hypoxia inhibits VEGF-mediated signaling pathways in cultured human endothelial cells (60), and VEGF expression is relatively low in ARAS (5). Under some conditions, hypoxia inducible factors (HIFs) appear to play a protective role in acute renal injury and adaptation to hypoxia (37) by upregulating factors that have been shown to be cytoprotective in hypoxic renal injury, including VEGF (44), heme oxygenase-1 (HO-1) (2, 53), and erythropoietin (EPO) (76). Surprisingly, these HIFs appear to promote the development of renal fibrosis when hypoxia is sustained and severe (57).

Prolonged activation of HIF signaling in renal epithelial cells enhances maladaptive responses, which lead to fibrosis and further tissue destruction (36). This occurs first through direct regulation of fibrogenic factor like tissue inhibitor of metalloproteinase-1 (TIMP-1) (58) and connective tissue growth factor (CTGF) (40) or synergy with transforming growth factor-β1 (TGF-β1), which is a potent profibrotic factor (69). Second, HIF-1 can initiate hypoxia-mediated apoptosis by increasing the expression of Bcl-2 binding proteins (BNIP3 and NIX), thereby inhibiting the anti-apoptotic effect of Bcl-2, or by stabilizing wild-type p53 (35), a tumor suppressor protein that is a potent transcription factor that can activate target genes that initiate cell death (e.g., Bax) or cause growth arrest (e.g., p21) in response to stress or DNA damage (63). Thus chronic hypoxia may restrict compensatory angiogenesis and decrease its efficiency for restoring stenotic kidney perfusion. Although restoring blood flow to the kidney after revascularization alone may reverse hypoxia in some patients, it fails to improve kidney function consistently or reverse tubulointerstitial inflammation (68). Revascularization alone also fails to restore renal expression of VEGF and its receptors (VEGFR-1 and R2) or HIF-1α that are attenuated in

![CT angiographic images](https://www.physiologyonline.org/content/30/3/177/F1)

**FIGURE 1.** CT angiographic images
CT angiographic images (top) of the right kidneys illustrating three patients with 1) no renal artery stenosis, 2) moderate renal artery stenosis, and 3) severe renal artery stenosis. Below each at bottom are Blood Oxygen Level-Dependent (BOLD) MR images with corresponding R2* parametric maps illustrating higher fraction of axial images with elevated deoxyhemoglobin evident with progressively more severe disease. Figure is from Ref. 34 and used with permission.
ARAS. Recent experimental studies also identify microvascular changes distal to a stenosis in the renal artery (82). Cellular activation in the poststenotic kidney elicits release of growth factors and cytokines, with primary roles suggested for angiotensin II, transforming growth factor (TGF)-β, and endothelin-1 (41). These in turn can stimulate production and activity of growth factors that mediate renal tissue response to collagen deposition, extracellular matrix turnover, fibrosis, and angiogenesis. Excessive matrix accumulation finally leads to irreversible scarring (15, 49). Over time, rarefaction of the distal arterioles develops, associated with fibrogenesis and loss of viable function (9, 10). Remarkably, histological studies of human biopsy tissue indicate that changes in the contralateral non-stenotic kidneys are similar to those seen in post-stenotic ARAS kidneys. These observations are consistent with “cross-talk” signaling between kidneys and wider systemic effects of inflammatory signaling, in addition to the putative effects of systemic hypertension and atherosclerosis per se (74, 80). The exact roles and timing of the above-mentioned processes in ARAS remain to be determined. Identification of reliable biochemical markers indicative of activated, hypoxic injury pathways in ARAS may have important prognostic value and may improve patient-specific therapy, including service as a marker to predict outcomes after renal revascularization (16). Some authors propose to use the BOLD imaging, including the response to transport inhibitors such as loop diuretics, as a potential predictive biomarker of renal functional outcome following revascularization in atheromatous renovascular disease (16). Other markers of hypoxia including HIF, NGAL, and kidney injury molecule-1 (KIM-1) have been proposed as well (71).

Atherosclerosis and Microvascular Loss in ARAs

The atherosclerotic environment itself (produced experimentally by cholesterol feeding) leads to defective angiogenesis and microvascular changes that limit oxygen delivery to renal tissue, which exacerbate hypoxia, tubular dysfunction, tubulointerstitial fibrosis, and renal failure (46). Microvessels are responsible for delivery of blood to the renal parenchyma and have distinctive abilities to adapt to local metabolic demands, sustaining renal function in early stages of RAS. The integrity of the renal microvasculature plays an important role in determining the responses to revascularization (6, 50). Hence, alterations in microvascular structure or function lead to hypoperfused and hypo-oxy-
generated regions in the kidney, triggering matrix accumulation, interstitial fibrosis, and renal dysfunction (5). Several pathways may contribute to microvascular damage in the stenotic kidney, including oxidative stress, apoptosis, inflammation, and fibrosis. Interestingly, inflammatory pathways damage the microvasculature through a mechanism characterized by “defective angiogenesis.” Although inflammatory cytokines stimulate angiogenesis in early disease (8), neovessels are often leaky, allowing inflammatory mediators to extravasate, thereby promoting infiltration of inflammatory cells into the interstitium and aggravating renal parenchymal injury. Hence, defective angiogenesis also contributes to renal microvascular damage. Downregulation of angiogenic factors, possibly due to increased ROS abundance, is associated with decreased spatial density of cortical and medullary microvessels in the stenotic kidney, suggesting that ongoing new vessel formation constitutes a crucial process in preservation of the renal microvasculature (20, 26). Capillary rarefaction, driven by impaired endothelial cell proliferation, plays a major role in the progression of chronic kidney disease (5). Previous studies show that administration of angiogenic factors like vascular endothelial growth factor (VEGF) can both prevent and reverse stenotic-kidney microvascular damage and is associated with significant improvement in renal function (5, 7). Taken together, reductions in blood flow (and thereby tissue oxygenation) and the atherosclerotic environment participate in a transition from a hemodynamic disorder to a pro-inflammatory interstitial injury within the post-stenotic kidney.

Can Post-Stenotic Kidney Injury Processes be Reversed?

ARAS represents one specific clinical manifestation of systemic atherosclerosis. Recent treatment recommendations emphasize the need for vigorous atherosclerotic risk factor management, including blood pressure control, lipid management with statins, withholding tobacco use, and aspirin (66). Antihypertensive drug therapy, especially using agents that block the (RAAS) system, such as angiotensin-converting enzyme (ACE) inhibitor or angiotensin receptor blocker (ARB) therapy, is now effective in achieving goal blood pressure levels in most ARAS patients. Recent studies indicate that most patients tolerate these agents without difficulty (38), although an increase in serum creatinine can occur. Patients treated with RAAS blockade have reduced mortality (75). Some patients develop clinically important reductions in eGFR with RAAS blockade but can be treated successfully with these agents after correction of the ARAS with endovascular stenting (72). As noted above, prospective treatment trials using either surgical or endovascular revascularization fail to demonstrate reversal of kidney injury once established.

Recent studies suggest that intrarenal administration of endothelial progenitor cells (EPC) restores tissue integrity in the post-stenotic kidney in the swine model (12, 14). Moreover, delivery of EPC into the stenotic kidney at the time of revascularization preserves oxygen-dependent tubular function, suggesting a role for EPC in the treatment of ARAS.

**Figure 3.** Micro-CT images of the kidney in experimental ARAS

Micro-CT images of the kidney in experimental ARAS showing improved microvascular architecture in post-stenotic kidney treated with mesenchymal stem cell (MSC) in addition to renal revascularization. MSC, mesenchymal stem cell; ARAS, atherosclerotic renal artery stenosis; PTRA, percutaneous transluminal renal angioplasty. Figure is from Ref. 25 and used with permission.
function and microvascular architecture, and decreases inflammation and fibrosis. These data argue that cell-based therapy may provide effective adjunctive therapy to improve renal outcomes in RAS (20). When adipose-derived mesenchymal stromal/stem cells (MSC) are delivered into the renal artery alone or in conjunction with renal revascularization in experimental models, there is substantial recovery of renal hemodynamics and function, decreased inflammation, apoptosis, oxidative stress, microvascular loss, and fibrosis (19, 24, 25) (FIGURE 3). Recent studies using agents that target and stabilize mitochondria, such as SS-31 (Bendavia, Stealth Biopharmaceuticals), a novel tetrapeptide that inhibits mitochondrial permeability transition pore opening, demonstrate reduced apoptosis, oxidative stress, microvascular rarefaction, and ischemia-reperfusion injury in experimental models when infused at the time of revascularization (23). Daily subcutaneous administration of this compound also attenuates kidney damage and improves oxygenation, suggesting that mitochondrial dysfunction may participate in both acute and chronic injury pathways in experimental ARAS (21).

How can clinicians reverse inflammatory injury in patients with ARAS? For most patients, restoring kidney blood flow alone through revascularization fails to reverse inflammatory pathways (as reflected by cytokine signatures from the renal vein) or to improve GFR in the affected kidney (68, 78); in contrast, innovative cell-based therapies can reverse renal injury, and addition of endothelial progenitor cells (EPC), mesenchymal stem cells (MSC), or other drugs like Simvastatin can restore them to normal levels (13, 20, 23). Indeed, determinants of progressive renal injury from chronic renal ischemia are poorly understood. Better understanding of the regulation of immunological signals and fibrogenic pathways will be critical to limit inflammatory renal damage, beyond selecting patients for restoring vessel patency.

Perspectives in Humans

Taken together, these studies delineate a paradigm shift related to vascular occlusive disease affecting the kidney. Atherosclerotic vascular disease can produce a fall in blood flow and GFR to which the kidney can “adapt” up to a certain level without overt tissue hypoxia. As vascular occlusion exceeds a critical threshold leading to cortical hypoxia, inflammatory pathways and microvascular rarefaction supervene to modify a hemodynamic stress to an environment with ongoing tissue injury. Early recognition of renal artery stenosis may prevent these developments, but recent prospective trials fail to identify a specific benefit to renal revascularization (17, 78). As a result, more patients than before are identified when disease progression has produced hypoxic and microvascular changes in post-stenotic kidneys. Improved therapy for this disease will depend on identification of biomarkers indicative of the degree of actual tissue ischemia within an individual subject.

In some respects, vascular occlusion from ARAS represents a clinical model in which the initiating kidney injury can be removed by restoring vessel patency. Recovering microvascular integrity and tubular function likely will require additional measures that improve the milieu for tissue repair, a feature likely necessary for many forms of kidney disease. Experimental studies using agents targeting mitochondrial protection and/or maneuvers using EPC and/or MSC administration suggest that cell-based therapy may be capable of reversing pro-inflammatory stimuli (25) and fostering tissue repair. Clinical investigation of cell-based therapy in human subjects is sorely needed.

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