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Renovascular disease, microcirculation, and the progression of renal injury: role of angiogenesis

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END-STAGE RENAL DISEASE (ESRD) is a growing health problem in the adult U.S. population that consumed $23.9 billion in 2009, a cost that has almost doubled in the past 10 years (55a). The 2009 United States Renal Data System report shows that the presence of chronic kidney disease (CKD) in current Medicare patients reaches up to 16.2%, with an incidence of 5.8% per year depending on the ethnicity (55a). Although hypertension and diabetes are still the most common etiologies, the role of vascular nephropathies and renovascular disease (RVD), a progressive condition caused by narrowing of the renal arteries, as causes of CKD and ESRD is on the rise among the elderly population.

One of the main causes of chronic RVD is renal artery stenosis, affecting 18–40% of those patients older than 65 (26) and almost 70% of patients with coronary or peripheral atherosclerotic vascular disease (41). Mainly because of atherosclerosis (61), RVD has a prevalence that ranges from 6.8% (32) to 23% (22), and up to 15% of those patients will develop progressive deterioration of renal function that may eventuate in CKD or ESRD (26, 32).

A defective renal microcirculation, also known as microvascular (MV) disease is a prominent pathological feature in CKD, irrespective of the cause, and progresses as CKD evolves (30). In general, the microcirculation is constituted by those vessels between 0 and 200 μm, which are embedded within organs and are responsible for the distribution of blood within tissues. Those small vessels in the kidney include interlobar, arcuate, and interlobular arteries, and smaller branching order microvessels like arterioles, capillaries, and venules. Partly mediated by augmented vasoconstriction and endothelial dysfunction in CKD (78), MV disease can alter renal blood flow and lead to a progressive decrease in peritubular capillary flow and consequently, mild tubulo-interstitial ischemia (63). Renal ischemia could be observed both as a cause and a consequence of damage in the kidney exposed to chronic RVD (46). An ischemic insult is a powerful stimulus to trigger renal neovascularization, a major physiological response that involves a sequence of events resulting in development of new vessels from preexisting ones. Vascular proliferation in the kidney not only occurs during the developmental stages of the organ, but is also a crucial mechanism by which the kidney faces a diversity of insults (8, 36, 72, 77). Nevertheless, RVD is characterized by intrarenal MV abnormalities that possibly aggravate the effects of the vascular obstruction in the main renal artery and exacerbate the progression of renal injury (6,
13, 49). It is possible that the severity and persistence of damage in the intra-renal MV bed may explain why the function of the stenotic kidney is not always restored (only 30% of reperusions are successful) and sometimes even continues to deteriorate after renal revascularization, the most frequent therapeutic approach in patients with chronic RVD.

The purpose of this review is to discuss the impact of the functional and structural changes of the renal microcirculation and the role that such changes may play in the progression and often irreversibility of renal injury in chronic RVD. I will also discuss the potential mechanisms of renal MV injury in the stenotic kidney and the feasibility of potential targeted therapeutic interventions.

**MV Disease and Renal Responses to Revascularization: the Missing Link?**

Restoration of tissue blood flow to the site of ischemic injury is crucial for developing a successful repair response. One of the most frequent therapeutic approaches to treat the chronically stenotic kidney in humans is by attempting restoration of blood flow either by opening the stenotic renal artery via renal angioplasty or through bypass surgery. While the renal vascular obstruction may represent a relatively straightforward problem to be addressed by revascularization, the optimal therapeutic approach to treat RVD is still not defined. Indeed, there is a significant gap between the technical success of revascularization (achieved in almost 100% of the cases) (73) and the resulting improvements in renal function or resolution of hypertension (25–30%) (66, 67). The severity of renal artery stenosis and the degree of impairment of renal function at the time of reperfusion may predict the relatively poor responses to revascularization (19, 20), but the mechanisms behind the progressive deterioration of renal function despite successful correction of the renal vascular obstruction are still unclear. Since generation of new vessels in the kidney seems to be a mechanism to preserve renal function activated in response to injury (14, 36, 76), it is possible that the damage of the renal MV architecture and deterioration of the angiogenic response constitute the early steps that lead to progressive renal injury. The extent, severity, and progression of MV damage in the ischemic renal parenchyma may play a pivotal role in determining the success of revascularization and explain why the ischemic kidney sometimes does not improve or continues to deteriorate even after restoration of blood flow. The factors determining these relatively poor outcomes, however, are still unknown, and advances in this field to understand the underlying mechanisms of irreversible renal injury are in dire need.

**Potential Mechanisms Underlying Renal MV Disease**

MV networks have the ability to adapt to the local metabolic tissue requirements by upregulating or downregulating angiogenesis, a process known as MV plasticity (50). These responses involve the recruitment and activation of numerous factors intertwined in a complex mechanism that ultimately preserve the MV architecture and function. We will briefly discuss them, as well as their role in inducing MV disease.

Ischemia: role of vascular endothelial growth factor. Thinking of ischemia as a key player in renal MV injury in RVD seems obvious. Although renal oxygen supply is one of the highest in the body, and only 10% of delivered oxygen is needed for metabolic demands, experimental evidence has shown that renal artery stenosis can decrease cortical and medullary oxygenation in the stenotic kidney (40, 58, 70). Facing a decrease in blood supply, the expected physiological response to ischemia, as may occur in chronic RVD, could include generation of new vessels to sustain tissue perfusion. A key player in MV proliferation and repair is vascular endothelial growth factor (VEGF), which shows different patterns of expression and availability depending on the ischemic environment. VEGF is crucial for preserving the microvasculature, in general, and operates in concert with other factors to promote cell division, migration, endothelial cell survival, and tube formation that ultimately generate, repair, and maintain MV networks, including those in the kidney.

VEGF increases significantly in cells exposed to acute hypoxia (55), but eventually decreases when hypoxia is prolonged (57), suggesting a biphasic regulation of VEGF and implying that cells releasing VEGF themselves are injured or unable to
secrete this cytokine. In vivo experimental and clinical evidence correlates with the in vitro studies, showing that chronic reductions of renal blood flow (14, 37), as occurs in RVD, progressive glomerulopathies (60), and CKD (30), are associated with significant reduction in VEGF. A recent clinical study underscores the progressive nature of renal MV disease in CKD, revealing marked deficiencies in VEGF in these patients accompanied by defective vascular repair, impaired angiogenic responses, and enhanced vascular injury (30). We have previously shown that chronic experimental RVD induces renal MV rarefaction and decreases the expression and availability of renal VEGF (14, 37, 76). Interestingly, these are also accompanied by decreased expression of downstream mediators of VEGF, such as angiopoietin-1 and Akt, suggesting a downregulation of the angiogenic cascade in the stenotic kidney (Fig. 1). The mechanisms for VEGF reduction in the stenotic kidney are not entirely clear, but they appear to be a combined result of altered post-transcriptional mechanisms (37) and a progressive loss of the sources (24, 56) of this angiogenic cytokine. The importance of VEGF for protecting the renal vasculature is further underscored by studies from our laboratory (7, 37) and others (42, 43), showing that intrarenal administration of VEGF in RVD preserved the renal MV architecture and function, improved renal blood flow and filtration function, and decreased renal fibrosis. More importantly, we have also shown that preserving the structure of the intrarenal microcirculation by this intervention is functionally consequential, since it significantly improved the renal responses to revascularization by renal angioplasty (7), supporting the key role of MV disease in determining the fate of the ischemic kidney.

Inflammation

Inflammation is a prominent injurious mechanism activated in CKD. Chronic inflammation has classically been shown in pathological situations, such as rheumatoid arthritis, diabetes, and cancer (23, 47), which also display increased angiogenesis in an exacerbated manner. Inflammatory cells can directly release angiogenic factors, such as VEGF, basic fibroblast growth factor, and tumor-necrosis factor (TNF)-α, among many others, at inflammatory loci, which put forth mitogenic and migratory effects in the endothelium, eventually promoting vascular proliferation (23).

We have recently shown that cardiovascular risk factors, such as lipid abnormalities (8, 12) and obesity (36), which are also key risk factors for renal disease, are associated with increased renal expression of TNF-α and significant renal cellular inflammatory infiltrates. TNF-α is a pivotal proinflammatory mediator that also stimulates vascular proliferation directly and by close interactions with other angiogenic cytokines, such as VEGF, nuclear factor-κB, and interleukins (23). The increased renal inflammation in these studies was accompanied by augmented renal MV density, likely reflecting a compensatory mechanism that sustained renal function in the early stages of both diseases. This concept was confirmed by
arresting TNF-α-induced angiogenesis (8), which diminished MV density and decreased renal blood flow and filtration function. Hence, inflammation-induced angiogenesis seems to be an early but partially protective compensatory mechanism, since the degree of renal injury remained unchanged [reflected by the significant tubulo-interstitial and glomerular damage in these models (8, 12, 36)] and likely progressive as the disease evolves. The notion that these newly generated vessels in chronic inflammatory milieux may promote the progression of tissue damage is observed in the kidney and also in other diseases as well (21, 45, 64). Inflammatory induced angiogenesis may indeed result in highly permeable, leaky neovessels that allow injurious cytokines to migrate to the extracellular space, thus promoting tissue injury (Fig. 2).

Renal inflammation is also increased in experimental RVD (10, 11) and could trigger MV proliferation in the stenotic kidney. Clinical and experimental studies have shown that the decline in renal oxygenation precedes inflammation, fibrotic changes in tubulo-interstitial cells, and matrix accumulation, suggesting that hypoxia may both initiate and promote the renal tissue damage (29). A chronic, sustained decrease in blood and oxygen supply in the stenotic kidney may then activate hypoxia-induced factors (36, 38), which, in turn, can promote inflammation and angiogenesis (38). However, despite renal inflammation, the stenotic kidney shows a marked reduction in MV density. This does not rule out the deleterious role of inflammation in aggravating renal injury and contributing to the progressive fibrosis and deterioration of renal function in the chronically stenotic kidney (10, 11). Since MV density is significantly diminished in the stenotic kidney, the chronic ischemic insult may surpass the proangiogenic phase of inflammation in the stenotic kidney, possibly activating antiangiogenic mediators (68) that accelerate the progression of renal MV rarefaction. It is possible that chronic hypoxia may potentiate inflammation and further contribute to extracellular matrix accumulation and increase renal fibrosis, in the absence of vascular regeneration and combined with MV dysfunction as occurs in the stenotic kidney.

Fibrosis

Irrespective of the etiology, renal fibrosis is the common final stage of progressive renal disease. Pivotal renal profibrotic factors often involved in chronic renal disease, such as transforming growth factor-β (28, 74) or connective tissue growth factor (5), have potent effects in stimulating angiogenesis. However, although we have shown that such factors are also upregulated in the stenotic kidney (10, 11, 15), their increase is accompanied not only by marked fibrosis, but also a significant MV rarefaction. The accumulation of extracellular matrix in the fibrotic kidney not only represents a buildup of scar tissue, but also generates an active source of potential angiogenic mediators, such as angiostatin, a potent inhibitor of VEGF and downstream mediators (65, 75). Angiostatin has been shown to be persistently elevated after ischemic renal injury and can significantly reduce VEGF-induced proliferation and repair of peritubular capillaries, hence accelerating tubular and interstitial damage (52). Other potent extracellular anti-angiogenic factors and inhibitors of cell proliferation that are highly expressed in kidneys are the thrombospondins (35), which we have shown to be augmented in the atherosclerotic kidney (9). In addition, we have previously shown that key enzymes for matrix degradation and removal and MV development, such as the matrix metalloproteinases-2 and -9, as well as the antifibrotic and proangiogenic hepatocyte growth factor, are reduced in the RVD kidney (11, 15). Decreased expression and activity of these factors lead to further accumulation of extracellular matrix, facilitating the buildup of intrarenal fibrosis and feeding a vicious circle (Fig. 3). In addition, the development of renal scarring involved progressive changes and likely loss of podocytes (27, 54), one of the main source of VEGF (24), thus, in turn, further contributing to this deleterious mechanism.

MV remodeling correlates with renal scarring (44), which, in turn, may subsequently invoke additional changes in vascular morphology, such as an increase in MV tortuosity (77), which may reflect the abnormal expansion and development of the renal vasculature against fibrotic tissue (Fig. 4). Overall, all these changes may further constrain and limit the already diminished MV proliferation, growth, and development in the stenotic kidney, ultimately aggravating MV rarefaction and consequentially renal injury. Hence, timely interventions to preserve a healthy intact microcirculation would likely interrupt this injurious feedforward mechanism that precipitates the progression of renal injury.
Therapeutic Approaches

Considering that mechanisms controlling the generation of new vessels may be exhausted, negated, or defective during sustained and progressive renal injury, targeted interventions to preserve the renal microcirculation may not only decrease the evolving injury in renal vascular disease but may also constitute a neoadjuvant intervention to improve the success of current strategies to improve renal function, such as revascularization. It has been shown that frequently used drugs in humans like statins (14, 18, 71), angiotensin (62), and endothelin (9, 39) receptor blockers, may regulate angiogenesis in different vascular beds, such as in the heart, brain, and kidney. However, those effects on the small vessels are reported mainly as collateral rather than as a main effect. There is a relative lack of targeted interventions on the renal microcirculation, and in this section, I briefly discuss promising evidence for such treatments.

Few and small human studies have tested the efficacy of proangiogenic therapies by direct administration of angiogenic cytokines (34), but the evidence supporting the administration of angiogenic factors comes mostly from studies in experimental settings (1, 33, 48). However, the use of angiogenic modulators to treat the ischemic kidney is virtually unexplored. The VEGF pathway has been a target for investigation as a proangiogenic therapy in ischemic settings (4, 17). We have recently demonstrated, in proof-of-concept studies, distinct vasculo- and renal-protective effects of intrarenal administration of VEGF in the stenotic kidney (7, 37). Although by experimental design, these effects were largely preventive and at a very early stage of the disease, the data were promising and support the necessity of further studies to fully characterize the potential of such intervention for human disease. Challenges for future studies and therapeutic intervention are that the onset of RVD in humans is rarely definable, the patients are diagnosed at different stages of the disease, and the severity and extent of renal compromise varies.

Significant attention has been directed to the biological and therapeutic capabilities of progenitor cells, an emerging field of research to treat ischemic tissues. The vascularendothelium is constantly exposed to mechanical and chemical insults, and the continuous process of repair is mediated, in part, by adjacent cells or by recruited progenitors. Circulating cells play a critical role in healing the endothelium when the intrinsic system is unable to adequately support tissue repair. Endothelial progenitor cells mobilized in response to ischemia play a crucial role in augmenting neovascularization of ischemic tissues and endothelial replacement after vascular injury. Defects in the number and/or function of endogenous cell progenitors have been observed in patients with coronary artery disease and diabetic nephropathy (25, 53, 59). Furthermore, clinical and experimental studies indicate that the number and function of circulating progenitors are also decreased in chronic renal disease (31, 51). Recent evidence suggests potential for cell-based repair in the acutely injured kidney to augment local regeneration, and this therapeutic approach to treat the kidney is gaining momentum. We have recently shown that the chronically stenotic kidney has a defective repair response to ischemia, since cell progenitors and the kidney showed abnormal
expression of homing cell-recruitment factors, hence, resulting in abnormal angiogenesis and MV rarefaction (16) . Although retention of cells in the renal tissue is low, the administration of cell progenitors likely leads to autocrine and paracrine effects on the surrounding cells. These cells reduce renal damage via secretion of angiogenic growth factors that induce mobilization of endogenous progenitor cells, which can then migrate and differentiate into mature vascular endothelial cells, promoting angiogenesis. In other words, this approach recuperates a renal endogenous vasculo-protective mechanism, since intrarenal administration of autologous endothelial progenitor cells significantly reversed MV rarefaction in the stenotic kidney, attenuated renal dysfunction, and decreased fibrosis (13).

**Conclusion, Perspectives and Significance**

Identifying means to determine the frontier between reversible and irreversible renal injury and when renal function may still be salvageable would have significant clinical impact. Future studies concentrating on noninvasive assessment of the renal architecture, and the response to established and novel treatment options should help on achieving such goals. The damage of the renal MV architecture and deterioration of the angiogenic response may constitute early crucial steps in the complex multiple pathways involved in progressive renal injury, and as the damage of the renal parenchyma progresses, the injury advances toward irreversibility. We have shown that a decreased cortical MV density in the chronic RVD kidney is associated with decreased renal blood flow, glomerular filtration rate, perfusion, and tubular function (10, 13, 14, 36). Such decreases in the microvasculature of the stenotic kidney affect interlobar, arcuate, and interlobular arteries, and smaller branching order microvessels like arterioles, capillaries, and venules. These deleterious changes in the MV architecture and function initially compromise the renal cortex but also extend at the later stages to the medulla (10, 36), as kidney disease evolves in our model. Our previous studies have also demonstrated a substantial deterioration of mainly proximal tubular and Henle’s loop function, as well as tubular atrophy (10) accompanying MV dysfunction, damage, and loss, indicating a vascular-tubular damage correlation (3). It is important to emphasize that experimental evidence from our laboratory supports the notion that the extent of the overall renal functional and structural damage could partly be reversible by targeted interventions that protect the renal microvasculature (13, 37). Do nephrons grow back or generate by such interventions? Likely not, but it is possible that restoration of blood flow by generation of new vessels that shunt preexisting damaged ones and MV repair that is led by such interventions also contributes to restore filtration function in partly damaged or hibernated (19, 69) but potentially recoverable nephrons.

An intact and healthy microcirculation is vital to restore blood flow to the injured tissues to initiate a successful repair response. Modifications in the renal microcirculation and generation of new vessels in the kidney seem to be processes activated in response to injury to preserve renal function, but a deregulated or insufficient formation of new vessels could be deleterious (9, 13, 14, 76). It is possible that, as glomerulo-sclerosis and tubulo-interstitial fibrosis represent the final stage of CKD, the deterioration of MV function, MV damage, and later MV loss may indeed represent the initial steps of renal injury in RVD. The resulting tissue ischemia thus serves as a stimulus for the activation of proinflammatory and profibrotic factors, leading to renal parenchymal injury and initiating a potential vicious circle that results in progressive, irreversible renal injury (Fig. 5). Hence, it is possible that current failures of therapeutic interventions in chronic RVD might be partly due to an overlooked or underestimated severity of renal MV disease at the moment of treatment.

Targeted interventions to enhance endogenous renoprotective mechanisms, such as cell-based therapy or the use of angiogenic cytokines have shown promising results in experimental animals and in some clinical settings. However, carefully designed prospective experimental and clinical studies are needed to determine the appropriate utilization of such therapeutic options, possibly accompanying established interventional techniques to ultimately improve the outcomes of patients with chronic renal disease.
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