Atherosclerotic renovascular disease (ARVD) is more commonly identified than ever before, particularly among the elderly population. By some accounts, >10% of new patients entering dialysis programs in the United States have ARVD as a major contributor to their end-stage renal disease. ARVD is also associated with accelerated renal injury, hypertension, and cardiovascular complications. Renovascular hypertension (RVH) is an independent cardiovascular risk factor and induces greater decline in cardiac function and structure than essential hypertension.1

However, clinical trials show consistently that revascularization of the stenotic renal artery using percutaneous transluminal renal angioplasty (PTRA) results in limited and inconsistent recovery of renal function.2 The reasons for the failure of revascularization to resolve renal dysfunction are complex and may include procedural complications, as well as pre-existing parenchymal injury, including endothelial dysfunction, inflammation, fibrosis, and microvascular remodeling in the poststenotic kidney.3

Atherogenic comorbidities constitute important catalysts of renal damage in ARVD. In fact, atherosclerotic nephropathy can produce renal damage even with hemodynamically insignificant stenoses,4 suggesting a direct effect of atherosclerosis on the kidney. Furthermore, unlike patients with fibromuscular dysplasia, in patients with ARVD a decrease in stenotic-kidney perfusion does not correlate with the angiographic degree of stenosis,5 and cortical perfusion is reduced even with mild stenoses, underscoring the contribution of additional pathogenic factors to renal damage. Therefore, the quest for underlying mechanisms and promising interventions mandates development of experimental platforms that not only involve renal artery stenosis but also emulate the atherosclerotic milieu.

Experimental Models of Renovascular Injury

Useful models of renal artery stenosis have been achieved in many laboratory animal species, including mice, rats, hamsters, rabbits, dogs, and monkeys, but usually without application of comorbidities.6 Our studies focused on developing a swine model with gradually developing renal artery stenosis secondary to unilateral renal arterial implantation of a local irritant coil. The advantages of the pig include its physiology, pathophysiology, vascular lesions, and lipid profile that resemble those observed in humans, and a body size suitable for use of clinical devices and interventions, and in turn rapid clinical translation.7 Pigs with renal artery stenosis are then fed with a high-cholesterol diet8,9 to simulate early diffuse atherosclerosis.

Our studies showed that atherosclerosis superimposed on renal artery stenosis exacerbates renal inflammation, oxidative stress, and fibrosis.8,9 Interestingly, development of RVH was not exacerbated, possibly partly because of increased sodium excretion in hypercholesterolemia.10 Furthermore, diffuse atherosclerosis aggravated injury in the contralateral kidney11,12 and the heart.13,14 Notably, RVH superimposed on hypercholesterolemia15 or diabetes mellitus16 exacerbates damage in the systemic vasculature and nonstenotic kidney in mice; further studies are needed to determine their impact on the murine stenotic kidney.

Studies using the ARVD swine model aimed to elucidate and target the mechanisms implicated in renal injury in ARVD. In 2-kidneys,1-clip rodents, RVH and renal injury are sustained by interactions among the rennin–angiotensin–aldosterone system, nitric oxide, and vasoconstrictor prostaglandins,17–20 which activate proinflammatory, prooxidant, and profibrogenic mechanisms.16,21 In ARVD these mechanisms are compounded by atherogenic factors, which intensify within the poststenotic kidneys, oxidative stress and inflammation22–24 and in turn rarefaction of microvessels.25–27 Loss of microvessels (Figure [A]), a hallmark of many renal diseases, may be driven by fibrosis-restricting expansion of the renal microcirculation,28 by microvascular regression, direct mechanical, or metabolic injury to the microvascular wall, or degradation of growth factors by reactive oxygen species.29,30 Apoptosis and mitochondrial injury also contribute to vascular loss, tubulointerstitial hypoxia, and interstitial fibrosis11,12 leading to renal dysfunction and scarring,27,33–38 the apparent irreversible phase of kidney damage.

The failure of renal artery revascularization to restore renal function in ARVD provides the impetus to explore underlying mechanisms and treat the poststenotic kidney directly. Given the postulated paradigm (Figure [B]), we attempted to address mechanisms recognized to induce kidney damage in ARVD.

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Mechanisms Underlying Poststenotic Kidney Injury

Hypoxia
The contribution of hypoxia to poststenotic kidney injury has been controversial, partly because early measurements of renal-vein oxygen pressure failed to identify a fall in PO$_2$. Nonetheless, studies in a rat 2-kidneys,1-clip model clearly documented renal hypoxia and inefficient oxygen utilization linked to increased oxidative stress. Indeed, careful studies of renal oxygenation indicate that renal vein PO$_2$ may be deceptive, insofar as it is affected by arteriovenous shunting, countercurrent exchange, and reduced oxygen consumption. Subsequently, oxygen probes and blood oxygen level–dependent MR have shown that renal hypoxia could be regional, and that significant stenoses induce hypoxia and fibrosis in poststenotic human kidneys.

Nevertheless, restoration of renal arterial patency in ARVD, which aims to alleviate hypoxia, does not necessarily restore stenotic kidney blood flow and function. In pigs with nonatherosclerotic renal artery stenosis, renal function and blood pressure promptly improved after PTRA, whereas in pigs with ARVD kidney dysfunction persisted, and correlated with microvascular remodeling, resembling findings of clinical trials. Incomplete recovery of stenotic kidney glomerular filtration rate (GFR) in pigs is linked not only to structural remodeling of the poststenotic kidney but also to preprocedural inflammation and the functionality of renal microvessels. Similarly, the poststenotic human kidney releases inflammatory mediators, which remain elevated after PTRA despite a fall in renal hypoxia. Therefore, although hypoxia characterizes severe renal artery stenosis, it may not be fully responsible for poststenotic kidney dysfunction. This notion may partly account for the inconsistent role of hypoxia in kidney injury in ARVD.

Atherosclerosis
To attenuate atherogenic factors such as hypercholesterolemia, we treated pigs with the cholesterol-lowering drug simvastatin. Simvastatin-treated pigs with renal artery stenosis and normal cholesterol improved stenotic kidney microvascular density and decreased glomerulosclerosis, despite unaltered cholesterol or blood pressure levels. Furthermore, in hypercholesterolemic ARVD pigs, despite unchanged RVH or cholesterol levels, simvastatin decreased renal oxidative stress and fibrosis, although GFR was unaffected. Hence, although HMG-CoA (3-hydroxy-3-methylglutaryl coenzyme-A) reductase inhibitors have considerable pleiotropic effects on the stenotic kidney, this regimen alone does not suffice to restore renal function.
Activation of the Renin–Angiotensin–Aldosterone System

Blockers of the renin–angiotensin–aldosterone system acutely decrease poststenotic kidney GFR, yet many patients with ARVD tolerate long-term treatment with these agents without difficulty. To explore direct effects of an angiotensin receptor blocker on the poststenotic kidney, pigs with renal artery stenosis were treated with either valsartan or triple therapy (reserpine/hydralazine/hydrochlorothiazide). For a similar decrease in blood pressure, valsartan did not impair GFR more than triple-therapy in this model. Both regimens increased microvessel density, but valsartan also improved poststenotic cortical perfusion and decreased oxidative stress. We interpret these data to indicate that direct beneficial effects of valsartan on the kidney parenchyma may offset its vascular effect to reduce GFR, thereby preserving kidney function. Clearly, inhibition of the renin–angiotensin–aldosterone system bestows blood pressure–independent benefits on both the stenotic kidney and the other target organs.

Oxidative and Inflammatory Injury

Angiotensin-II is a potent activator of NAD(P)H oxidase, a major enzymatic source for reactive oxygen species, and other inflammatory and athereogenic mediators increase reactive oxygen species production in the kidney and target organs. In pigs with ARVD, we blunted oxidative stress using a combination of the common antioxidants vitamins E and C. Antioxidant vitamins had no effect on blood pressure or GFR in pigs with renal artery stenosis, yet decreased renovascular resistance, improved tubular fluid dynamics, and decreased inflammation and fibrosis. Similarly, the antioxidant tempol effectively increases cortical blood flow and oxygen tension in clipped kidneys of 2-kidneys,1-clip rats. In pigs with ARVD, antioxidant vitamins improved endothelial function and upregulated protein degradation systems, resulting in blunted glomerulosclerosis and renal fibrosis, although GFR was not restored in this model. These studies positioned oxidative stress as an important mediator of renal injury in ARVD. The effects of antioxidant are partly mediated by their ability to attenuate renal remodeling because they are more effective in improving renal function and structure when delivered chronically than acutely. Antioxidants also restored microvascular architecture in the kidney and myocardium in renovascular hypertension, but did not fully restore GFR. Negative results of clinical trials also argued against their widespread use. Indeed, in healthy pigs, antioxidant vitamins led to a paradoxical increase in oxidative stress. Therefore, alternative strategies are needed to preserve poststenotic kidney function.

Because monocyte chemoattractant protein-1 expression is upregulated in the stenotic kidney in both pigs and mice, we postulated that this inflammatory mediator contributes to poststenotic kidney injury. In pigs, inhibition of monocyte chemoattractant protein-1 with Bindarit has no effect on RVH but improves renal blood flow, GFR, and endothelial function. Tubulointerstitial oxidative stress, inflammation, and fibrosis decreased, although microvascular density increased only slightly. Hence, monocyte chemoattractant protein-1 contributes to functional and structural remodeling of the poststenotic kidney and its inhibition may be protective.

Microvascular Rarefaction

Regression and remodeling of intrarenal microvessels are pivotal mechanisms responsible for loss of poststenotic kidney function and progression of chronic kidney disease, and delivery of vascular growth factors improves renal function. Similarly, antagonism of the endothelin-A receptor enhances microvascular density and renal recovery in pigs with renal artery stenosis, ascribing an important role to endothelin-1 in regulating the microcirculation in the poststenotic kidney.

Microvascular rarefaction is also amplified by impairment of endogenous repair mechanisms. Circulating endothelial progenitor cells (EPCs) are recruited in response to injury signals to facilitate microvascular and cellular repair, but their number and function can decline in subjects with ARVD after exposure to cardiovascular risk factors. We reasoned that replenishing EPC might enhance repair in poststenotic kidneys.

We isolated from peripheral blood endothelial outgrowth progenitors, which possess proangiogenic characteristics. Injecting these cells directly into the stenotic renal artery increased renal expression of angiogenic factors, enhanced proliferation and maturation of vessels, attenuated microvascular remodeling and fibrosis, and ultimately improved renal blood flow, endothelial function, and GFR. EPC delivery also protects the murine kidney subjected to acute ischemic injury. Their effectiveness in ARVD is enabled by the expression of cognate receptors corresponding to injury signals released from the stenotic kidney, facilitating their local adhesion and retention. Thus, autologous EPCs are effective in both nonatherosclerotic renal artery stenosis and ARVD.

Interestingly, the potency of EPC is greatest at the early phase of renovascular hypertension, corresponding to activation of the renin–angiotensin–aldosterone system. Yet, EPCs are less effective in the renal medulla than in the cortex, possibly because few EPCs reach this region. Nonetheless, tubular function was normalized and fibrosis attenuated when EPCs were delivered in association with PTRA. Adjunctive EPC delivery during PTRA restored GFR, improved renal blood flow and microvascular density, and decreased prevalence of proinflammatory macrophages and cytokines. Therefore, EPCs decrease poststenotic kidney injury and improve revascularization outcomes by preserving microvascular architecture and function and decreasing inflammation and fibrosis. Notably, preservation of renal function in turn improves myocardial microvascular integrity, underscoring the important contribution of renal dysfunction from ARVD to its cardiovascular sequelae.

However, EPCs are difficult to harvest from peripheral blood, and their immunogenicity mandates autologous administration. Emerging evidence shows that mesenchymal stem cells (MSC) are accessible for harvesting from many tissue sources, grow robustly in culture, and their anti-inflammatory and immunomodulatory properties render them potentially suitable for allogeneic applications. We found that MSC delivery in conjunction with PTRA in ARVD attenuated...
interstitial fibrosis, inflammation, microvascular remodeling, oxidative stress, and apoptosis, and restored stenotic kidney hemodynamics and function, underscoring their therapeutic potential. Importantly, MSCs are slightly more effective than EPC in restoring renal function, possibly because of greater potency in blunting inflammation and apoptosis. MSCs decrease stenotic kidney release of tumor necrosis factor-α and monocyte chemoattractant protein-1, consistent with their potent anti-inflammatory properties.

The mechanisms of action of MSC might include regulation of post-transcriptional regulators such as micro-RNAs and release of extracellular membrane vesicles. Micro-RNA and mRNA packed in these vectors modulate angiogenesis and other pathways in recipient cells and mediate the paracrine activities of MSC (Figure [A]).

Similar to EPCs, MSCs are less effective in repairing medullary damage, possibly because of their relatively lower engraftment rate compared with the cortex (=1.5). Yet, MSCs adjunctive to PTRA attenuate tubular injury and inflammation and reduce capillary loss. Alas, they may paradoxically increase medullary hypoxia by boosting tubular oxygen consumption.

Mitochondrial Injury

Ischemic kidney injury might be propagated by cell death and disturbed cellular energetics, events that are regulated by mitochondria. Furthermore, ischemia/reperfusion injury may contribute to limited efficacy of renal artery revascularization. To assess the involvement of mitochondria, we infused intravenously a mitochondrial-targeted peptide immediately before and during PTRA. Bendavia is a tetrapeptide that prevents oxidation of cardiolipin, a phospholipid constituent of the inner mitochondrial membrane. Stenotic kidney blood flow and GFR improved 4 weeks after PTRA with adjunctive Bendavia infusion, compared with pigs undergoing PTRA accompanied by vehicle infusion. Bendavia restored renal mitochondrial biogenesis and decreased microvascular refraction, apoptosis, oxidative stress, and fibrosis. Hence, mitochondrial injury incurred during reperfusion may amplify renal injury and limit recovery after PTRA. Importantly, chronic Bendavia treatment without PTRA was also effective in blunting renal damage in ARVD, normalized renal endothelial function, improved kidney oxygenation, and attenuated fibrosis. Studies in rats have demonstrated the efficacy of pretreatment with a mitochondrial-targeted peptide to increased renal tolerance to acute ischemia. These studies suggest that mitochondrial dysfunction contributes to the pathogenesis of both chronic and acute aspects of kidney injury, and targeting these organelles might protect the ischemic kidney (Figure [B]). Mitochondrial protection also improved cardiac function after reversal of RVH.

Systemic Effects of ARVD

An important observation is that pathways of injury in a stenotic kidney contribute to significant risks for the nonstenotic kidney, the cardiovascular system, and arterial pressure. The poststenotic kidney in both animals and human subjects releases inflammatory injury signals, and their levels rise not only in the stenotic but also in the contralateral kidney, suggesting renorenal crosstalk and systemic effects of ARVD. Consequently, damage and inflammatory markers in the contralateral kidney are greater than those induced by simple nephrectomy or even angiotensin-II infusion. Furthermore, cardiac hypertrophy and dysfunction are magnified in patients with RVH compared with essential hypertension.

Remarkably, the magnitude of RVH seems to be at least partly dissociated from the extent of renal injury. For example, in swine, ARVD-superimposed atherosclerosis magnifies renal injury, yet RVH levels remain similar to those in pigs with nonatherosclerotic renal artery stenosis. Furthermore, hypertension can be fully reversed by PTRA despite residual kidney injury, again suggesting that kidney injury is not closely correlated to the severity of RVH. The dissociation between the reversibility of renal function and hypertension in animal models is consistent with the results of the CORAL trial, in which despite the lack of efficacy of stenting to improve renal function compared with medical therapy alone, it achieved a modest decrease in blood pressure.

These observations underscore the need for multiple levels of therapy in this disease. Revascularization may have a role specifically related to blood pressure control and avoiding pulmonary edema, whereas adjunctive therapies, directly targeting underlying mechanisms responsible for renal injury in ARVD, are required to recover renal function. Identification of the basic mechanisms that lead to kidney tissue injury in ARVD can assist in the development of targeted therapies. We think that addressing those injurious pathways directly can improve stenotic kidney blood flow, GFR, fibrosis, and reverse microvascular loss. They may also improve the efficacy of revascularization with PTRA and stenting. Such targeted therapeutic interventions with or without PTRA might constitute effective tools for improving kidney and cardiovascular outcomes of ARVD. Importantly, some of the novel experimental approaches seem to also benefit the contralateral kidney and the heart in pigs with ARVD.

Building on observations in animal models, our group has recently moved forward to extend these paradigms into clinical trials for ARVD. We are currently studying the effects of intra-arterial delivery of adipose tissue–derived MSC on tissue oxygenation and inflammatory injury. For patients who are candidates for renal revascularization, phase I trials of intravenous Bendavia will allow direct examination of mitochondrial protection during ischemia/reperfusion processes in the poststenotic kidney during PTRA and stenting. These steps toward clinical translation may assist clinicians in developing strategies to protect and recover function in the poststenotic kidney.

Perspectives

The prevalence of ARVD is on the rise. The failure to demonstrate benefits from renal artery revascularization in prospective clinical trials has led to a precipitous fall in the application of PTRA and stenting, leaving few options available to preserve the poststenotic kidney. Alas, the presence of this kidney, by virtue of low function and robust cytokine
production, introduces a significant cardiovascular risk, underscoring the need to identify strategies to attenuate kidney injury and increase the efficacy of interventional techniques. To this end, relevant experimental models are proving invaluable to afford insights into the pathogenesis of ARVD and serve as experimental platforms to test novel therapeutic tools (Figure [B]). Further translational and clinical trials are needed to explore and develop innovative approaches to preserve the poststenotic kidney and improve the management of patients with ARVD.

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References


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