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Renal denervation prevents long-term sequelae of ischemic renal injury

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Signals that drive interstitial fibrogenesis after renal ischemia reperfusion injury remain undefined. Sympathetic activation manifests even in the early clinical stages of chronic kidney disease and is directly related to disease severity. A role for renal nerves in renal interstitial fibrogenesis in the setting of ischemia reperfusion injury has not been studied. In male 129S1/SvImJ mice, ischemia reperfusion injury induced tubulointerstitial fibrosis as indicated by collagen deposition and profibrotic protein expression 4 to 16 days after the injury. Leukocyte influx, proinflammatory protein expression, oxidative stress, apoptosis, and cell cycle arrest at G2/M phase were enhanced after ischemia reperfusion injury. Renal denervation at the time of injury or up to 1 day post injury improved histology, decreased proinflammatory/profibrotic responses and apoptosis, and prevented G2/M cell cycle arrest in the kidney. Treatment with afferent nerve-derived calcitonin gene-related peptide (CGRP) or efferent nerve-derived norepinephrine in denervated and ischemia reperfusion injury-induced kidneys mimicked innervation, restored inflammation and fibrosis, induced G2/M arrest, and enhanced TGF-β1 activation. Blocking norepinephrine or CGRP function using respective receptor blockers prevented these effects. Consistent with the *in vivo* study, treatment with either norepinephrine or CGRP induced G2/M cell cycle arrest in HK-2 proximal tubule cells, whereas antagonists against their respective receptors prevented G2/M arrest. Thus, renal nerve stimulation is a primary mechanism and renal nerve-derived factors drive epithelial cell cycle arrest and the inflammatory cascade causing interstitial fibrogenesis after ischemia reperfusion injury.

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Patients surviving an episode of acute kidney injury have a significant risk of progression to chronic kidney disease (CKD) and even end-stage renal disease.[1] However, the mechanisms that link acute kidney injury to CKD in humans remain poorly defined. Animal studies have shown that persistent medullary hypoxia, capillary rarefaction, inflammation, failed differentiation of epithelial cells, and apoptosis following renal ischemia reperfusion injury (IRI) are possible mechanisms that may drive tubulointerstitial fibrosis.[2–4] Several molecules induced after IRI are implicated in injury and inflammation, as well as in repair, regeneration, and in the progression of renal fibrosis. These molecules include various cytokines (interleukin-13 and interleukin-21), chemokines (KC, MIP-2, MCP-1), angiogenic factors (vascular endothelial growth factor), growth factors (epidermal growth factor, tumor growth factor-β1 (TGF-β1), connective tissue growth factor, platelet-derived growth factor), and the renin–angiotensin system.[5–7] The interplay of these molecules and their downstream signaling pathways in the injured or regenerating tubular epithelium, capillary, and interstitial cells could evoke inflammation, fibroblast differentiation, and proliferation and matrix deposition. However, the primary stimuli that induce the myriad of signaling events that lead to the inflammatory response and fibrosis after an initial insult to the kidney remains undefined.[8] Therefore, identification of the primary signal or the core signaling pathway that instigate renal fibrogenesis after an initial stimulus is essential for the elucidation of the pathophysiological mechanisms of the syndrome and in developing effective therapeutic strategies for preventing, reversing, or limiting progression of fibrogenesis.[9]

The kidney is innervated by efferent sympathetic nerves as well as peptidergic sensory afferent nerves in which several neuroactive substances have been identified.[10–12] Sympathetic nerve activity is increased in both patients and experimental animals with chronic renal failure.[13,14] Renal denervation shows protective effects against renal failure in both animals and humans. Although the mechanisms remain to be fully elucidated, it may include decrease in blood pressure, renal efferent sympathetic nerve activity, central sympathetic nerve activity and sympathetic outflow, and downregulation of the renin–angiotensin system.[12,15] Given the pronounced effect of

the renal nerves on CKD, we sought to determine whether afferent and efferent nerve-derived neuropeptides/neurotransmitters and their signaling pathways may be responsible for the functional, fibrotic, and inflammatory responses in IRI-induced long-term sequelae. Here we report that renal nerve-derived norepinephrine and calcitonin gene-related peptide (CGRP) signaling is required for tubular epithelial cell injury and production of inflammatory factors and profibrogenic factors to trigger renal interstitial fibrogenesis.

RESULTS

Renal denervation attenuates interstitial fibrosis induced by IRI

To test whether kidney nerve contributes to interstitial fibrosis induced by IRI, we performed renal denervation before IRI. Collagen deposition assessed by Sirius red staining and hydroxyproline measurement increased after IRI in intact kidneys in a time-dependent manner, whereas renal denervation markedly lessened the collagen deposition (Figure 1a and b, and Supplementary Figure S1A online). Renal denervation also reduced the myofibroblast marker α-smooth muscle actin expression, Smad3 phosphorylation (p-Smad3), and TGF-β1 production during interstitial fibrosis induced by IRI (Supplementary Figure S1B and C online). As inflammation has as an important role in fibrosis, we next examined the recruitment of neutrophils and macrophages. Polymorphonuclear neutrophil)-positive neutrophils and F4/80-positive macrophages were persistently recruited into intact kidneys for at least 16 days after IRI, whereas renal denervation inhibited the recruitment of both cell populations during interstitial fibrosis (Figure 1c–e). In addition, we determined that postconditioning of renal denervation (up to 1 day post injury) significantly reduced interstitial fibrosis and inflammation as demonstrated by decreased collagen deposition, profibrotic protein expression, and recruitment of neutrophils and macrophages (Supplementary Figure S2 online).

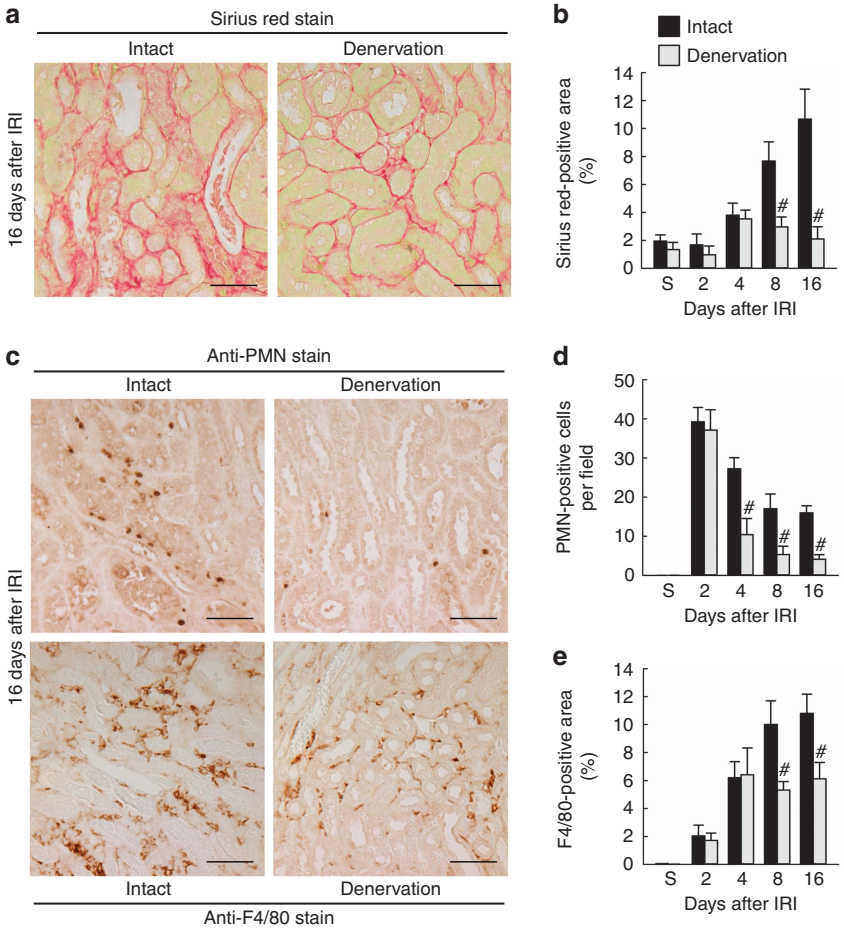

Figure 1 | Renal denervation inhibits collagen deposition, and neutrophil and macrophage influx during a period of interstitial fibrosis after ischemia reperfusion injury (IRI). Two days after denervation in left kidneys of mice, IRI or sham operation (S) in the left kidneys was carried out. (**a**) Collagen deposition using Sirius red stain on denervated or intact kidney sections at 16 days after IRI. Bar = 50 μm. (**b**) Percentage of Sirius red-positive area on kidney sections. (**c**, **d**) Neutrophil infiltration represented by the number of polymorphonuclear neutrophil (PMN)-positive cells on immunohistochemically stained kidney sections. (**c**, **e**) Macrophage infiltration represented by the percentage of F4/80-positive area on immunohistochemically stained kidney sections. Error bars represent s.d. ($n = 5$). $^{\#}P < 0.05$ versus intact. Bar = 50 μm.