Life and Death of Podocytes: Not Only a Matter of Vascular Endothelial Growth Factor

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Renovascular disease (RVD) comprises a variety of conditions that alter the arterial supply of the kidney, inducing hypertension and affecting renal function, ultimately leading to chronic kidney disease. Although renal artery interventions are frequent therapeutic strategies for treating RVD, many patients derive little net benefit from them. This creates an urgent need for the identification of disease mediators that could be targeted to prevent irreversible injury in the post-stenotic kidney or other complications.

Soon after its discovery, endothelin-1 (ET-1) was identified as a key factor in controlling kidney function and promoting renal disease progression [1], due to its vasoconstrictive action and mitogenic property. More recently, the role of ET-1 in fostering renal injury in chronic RVD was put forward, with a particular focus on the roles of ET receptors, ET-1 type A (ET_A) and ET-1 type B (ET_B) [2, 3]. Results from these studies documented the disparate effects of the selective antagonism of ET_A receptor compared to dual ET_A/ET_B [3] or ET_B receptor [2] blockade in a porcine model of chronic RVD. While ET_A receptor antagonist improved hypertension, microvascular rarefaction and renal injury, dual ET_A/ET_B blunted the therapeutic effects, and single ET_B receptor inhibition was ineffective.

In the current issue of the *American Journal of Nephrology*, Harvey et al. further investigated the involve-

ment of the ET-1 pathway in chronic RVD, examining the effects of the ET_A and ET_B receptor antagonism on podocyte integrity and survival in the ischemic kidney [4]. Podocytes are highly specialized epithelial cells constituting the outer layer of the glomerular filtration barrier, and their dysfunction and loss are hallmarks of proteinuric renal disease [5]. Notably, podocytes possess a fully functional ET system, and evidence has highlighted the role of ET-1 in promoting structural and functional alteration of these cells in renal disease [5]. Mindful of the previously reported in vivo effects of ET-1 blockade, in the present work, the authors exploited a hypoxic in vitro approach that offered the opportunity to dissect the mechanisms underlying podocyte injury and recovery by mimicking the renal environment of chronic RVD. They analysed human podocytes that were exposed to chronic hypoxia and treated with either an ET_A or ET_B receptor antagonist. Hypoxia decreased the podocyte count, while it increased the number of cells displaying morphological changes suggestive of apoptosis, such as nuclear condensation and fragmentation. These effects paralleled the decreased expression of vascular endothelial growth factor (VEGF) and slit diaphragm-associated proteins podocin and nephrin. Furthermore, hypoxic podocytes displayed increased expression of the anti-VEGF soluble receptor s-Flt 1 and of pro-apoptotic mediators, including BAX, p53 and SMAC. Treatment with ET_A receptor antagonist

significantly improved podocyte survival, preserved VEGF production and attenuated apoptotic factor expression, promoting instead a pro-survival expression profile with the upregulation of p-akt and Bcl-2. These effects were not observed after ET_B blockade, which conversely was harmful to hypoxic podocytes by inducing an increased s-Flt 1 secretion in the cell medium.

The first finding of this study is the beneficial effect of ET_A – not shared by ET_B – receptor antagonist on hypoxic podocytes. Although it is well known that ET-1 favours renal disease progression via the detrimental action of the peptide on podocytes, it is still a matter of debate whether selective ET_A receptor over ET_A/ET_B receptor blockade should be preferred for the treatment of kidney diseases. In an aging-associated glomerulosclerosis model, the ET_A selective antagonism was more effective than non-selective ET_A/ET_B receptor blockade in reversing glomerulosclerosis and attenuating podocyte structural damage [6]. Moreover, selective ET_A receptor and non-selective ET_A/ET_B receptor blockades reduced both albuminuria and glomerular permeability in a model of diabetic nephropathy, but only selective ET_A receptor blockade restored nephrin and podocin expression and decreased glomerular inflammation [7]. By contrast, results from transgenic mice indicated that podocyte-specific deletion of both ET_A and ET_B receptors was protective in diabetic mice, offering the rationale for hypothesizing that dual ET_A/ET_B is more beneficial than single ET_A antagonism [8]. In such a controversial context, the new findings by Harvey et al. add support to the use of selective over non-selective ET-1 receptor blockade for the treatment of chronic RVD.

The second significant finding of this study relates to the mechanism underlying the ET_A antagonist-mediated podocyte protection. Taking advantage of the use of VEGF neutralizing antibodies, Harvey et al. identified VEGF as a pivotal factor for podocyte protection mediated by the ET_A receptor blockade, and proposed VEGF's scarce bioavailability as a potential explanation for the detrimental effects of single ET_B receptor inhibition [4]. Podocytes are both the source and target of glomerular VEGF, which is essential for maintaining the normal podocyte function and phenotype. Among these, an elegant study by Eremina et al. [9] clearly demonstrated the protective role of podocyte-produced VEGF in the pathogenesis of renal thrombotic microangiopathy, indicating the importance of preserving podocyte VEGF production for maintaining glomerular filtration barrier health and function. The ability of ET_A antagonist to affect VEGF production was previously described in animal models of

diabetes, but was confined only to the heart. In type I diabetes, the administration of ET_A antagonist normalized the downregulated levels of VEGF, and this paralleled the recovery of cardiac contraction [10], while in a model of type II diabetes, a significant induction of myocardial VEGF expression was obtained after ET_A antagonism treatment, and was associated with the restoration of the heart's capillary network [11]. Harvey et al. have now provided the first piece of evidence that ET_A antagonism can have a direct effect on glomerular podocytes through VEGF.

The in vitro approach of this study would represent a potential limitation to its clinical applicability. To this end, additional in vivo investigation is warranted to confirm that ET_A antagonism-mediated podocyte protection is due to VEGF. The unprecedented role of VEGF in mediating the protective effect of ET_A antagonism on hypoxic podocytes proposed by Harvey et al. makes it possible to envision the ET_A receptor/VEGF pathway involvement in other renal diseases characterized by oxidative stress and fibrosis, such as diabetes. Notably, in experimental diabetic nephropathy, a number of studies documented the synergistic renoprotective effect exerted by the association of ET_A receptor antagonism with angiotensin-converting enzyme inhibitors (ACEi) [11, 12]. Such positive preclinical results provided the rationale for current trials in patients. Thus, the investigation of whether the effects of combined ACEi – ET_A receptor antagonism treatment are related to VEGF production in patients in ongoing trials could be an intriguing way forward.

Disclosure Statement

The authors have no conflict of interest to declare.

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