ENDOTHELIAL FACTORS IN THE PATHOGENESIS AND TREATMENT OF CHRONIC KIDNEY DISEASE

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Kidney damage can be both a consequence and a cause of arterial hypertension and cardiovascular (CV) disease [1]. Chronic kidney disease (CKD) patients are, in fact, at increased risk of coronary and cerebrovascular events, the major causes of death even before the development of end-stage renal disease (ESRD) [1]. Hypertension implies a shift of the endothelium toward a proinflammatory, prothrombotic state. Considering the specific functions of glomeruli and tubules, endothelial dysfunction and/or loss translates into dynamic changes of filtration fraction, resulting in a progressive reduction of the glomerular filtration rate (GFR), extracellular fluid volume expansion, abnormal ion balance, and renal hypoxia, ultimately leading to CKD.

Recognizing the key role played by the endothelium in cardio-renal health, as well as the lack of updated information on its role in CKD, the Working Group on Endothelin and Endothelial Factors of the European Society of Hypertension in conjunction with the Japanese Society of Hypertension prepared a consensus document to review current knowledge of the mechanisms underlying endothelial cell injury and discuss its role in renal damage associated with arterial hypertension, diabetes mellitus, preeclampsia, kidney transplantation and cancer [2,3]. This Newsletter aims to provide a concise summary of this document [2,3].

Endothelium–dependent mechanisms in renal injury

High blood pressure-associated mechanical stress, alongside hypoxia, aging, smoking, hypercholesterolemia, diabetes mellitus, and hyperhomocysteinemia, increases formation of reactive oxygen species (ROS), which inactivate the vasodilating nitric oxide (NO) causing the formation of peroxynitrite (ONOO) and of vasoconstricting and mitogenic substances and an imbalance towards vasoconstriction and vascular remodeling. A decreased bioavailability of NO and/or an increased release of NO inhibitors, such as asymmetric dimethylarginine (ADMA), reduce NO bioactivity on the renal vascular smooth muscle cells (VSMC) blunting vasodilation (for a review see [2]). An impaired release of factors causing endothelium-derived hyperpolarization (EDH) (like epoxyeicosatrienoic acids), by reducing K^+ channels activity and promoting Ca^{2+} influx, also activates VSMC contraction. When the release of the endothelium-dependent vasodilators and EDH is blunted, angiotensin II (Ang II) and endothelin-1 (ET-1) act unopposed to induce renal and systemic vasoconstriction, thus causing reduced renal blood flow and hypoxia. The latter facilitates the formation of hypoxia-inducible factor-1 (HIF-1) and ROS that, via inflammatory nitric oxide synthase (iNOS), promote generation of large amounts of NO in leukocytes, VSMCs, and epithelial tubular cells, thereby generating peroxynitrite that aggravates the microcirculatory dysfunction and causes protein nitrosylation and thereby functional injury to proteins.

The production of ET-1, mostly triggered by Ang II, ROS, and pro-inflammatory cytokines, can occur not only in endothelial cells, but also in podocytes, parietal epithelial and mesangial cells. Endothelial cells secrete ET-1 predominantly abluminally, e.g. towards the vessel wall, where ET_A and ET_B receptors are located, thus acting in a paracrine fashion to enhance vasoconstriction and cell proliferation. However, by acting in an autocrine fashion on the ET_B receptor subtypes of endothelial cells, ET-1 releases NO and prostacyclin thereby counterbalancing vasoconstriction. In CKD plasma ET-1 is increased, likely because of augmented production and reduced clearance. Notably, urinary excretion of ET-1 increases as renal function declines, suggesting that activation of the renal ET system concurs with hypertension in worsening renal function.

The renin angiotensin aldosterone system (RAAS), ET-1 and NO interact in a complex fashion (Fig. 1): Ang II stimulates the release of ET-1 in endothelial cells, podocytes and mesangial cells, and upregulates the expression of the ET_A receptors. In contrast to Ang II, that vasoconstricts more the postglomerular
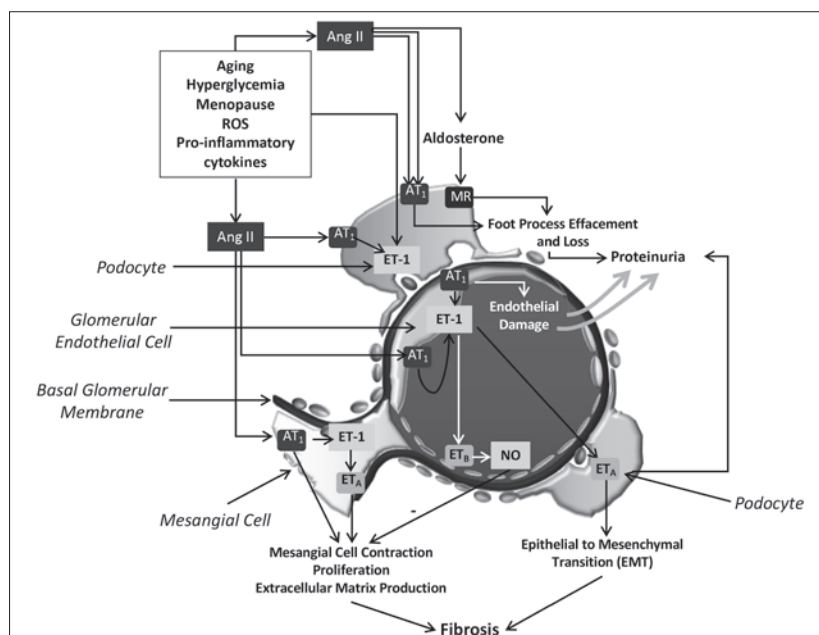

Figure 1. *Effects of the RAAS and ET-1 system in the kidney. Under physiological conditions Ang II maintains glomerular filtration rate by modulating the efferent arteriole tone, and regulates blood volume by stimulating water and Na^+ absorption in the distal and collecting tubules, directly or indirectly via aldosterone production. Aging, hyperglycemia, hypoxia and inflammation, characterized by release of ROS and cytokines, cause excess Ang II and ET-1 synthesis that induces constriction of both afferent and efferent arterioles with reduced GFR, and also reduction of NO° bioavailability [1].*

vessels, ET-1 causes mainly preglomerular constriction. Both peptides Ang II and ET-1 promote mesangial cell contraction and extracellular matrix production, and enhance tubular Na^+ absorption, an effect that is augmented by the secretagogue actions of both peptides on aldosterone, that by itself also induces renal vasoconstriction and microalbuminuria.

In transgenic rodents overexpressing renin that develop malignant Ang II-dependent hypertension, ET-1 promotes renal fibrosis by activating the epithelial to mesenchymal transition (EMT), a process whereby endothelial cells lose their phenotype and acquire that of mesenchymal cells, thus transforming into collagen-producing myofibroblasts. EMT leads to microvascular rarefaction and fibrosis, with consequent hypoxia that interferes with endothelial cell repair and regeneration [4].

Factors that injury endothelium also induce sulfation and deacetylation of heparan sulfate in the glycocalyx that, acting as lining of the endothelial cells, physiologically regulates vascular permeability. Damage of glycocalyx increases the expression of selectins and integrins promoting inflammatory pathways, and also activates heparanase and hyaluronidase II that degrade the glycocalyx itself, altering permeability with onset of proteinuria [5].

Diabetic nephropathy

Diabetic nephropathy is characterized by focal and segmental glomerulosclerosis that depends not only on hyperglycemia, but also on activation of endothelium-dependent mechanisms involving the RAAS and ET-1. Obesity, commonly associated with insulin resistance and/or diabetes, also leads to focal-segmental glomerulosclerosis, (FSGS), which in obese patients has been termed "obesity nephropathy".

Drugs targeting endothelial pathways, as angiotensin I converting enzyme (ACE) inhibitors, angiotensin AT_1 receptor blocker (ARBs), mineralocorticoid receptor antagonists, and in part also ET-1 receptor antagonists (ERAs) [6], were shown to improve clinical outcome with benefit that exceeded that attributable to changes in blood pressure (for a review see [3]).

Kidney transplantation not only normalized BP, but also reversed cardiac and retinal damage, in patients with proteinuric renal disease, indicating that end-organ injury can be regressed. Similarly, regression or partial remission of proteinuria occurred in patients with diabetic nephropathy treated with ARBs or ACE inhibitors and, occasionally, with ET-1 receptor antagonists (ERA) (see [3]).

Preeclampsia

Preeclampsia is characterized by an increased production of placental factors, as soluble Fms-like tyrosine kinase 1 (sFlt-1), soluble endoglin, agonistic auto-antibodies to the AT_1 receptor, and inflammatory cytokines, which contribute to generalized endothelial dysfunction [7,8]. These factors not only affect the growth of the placenta and foetus, but also the glomerular filtration barrier and the balance between pro- and anti-angiogenic factors (see [3]).

Kidney transplantation and transplant–associated hypertension

Oxidative stress, which is enhanced before transplantation during ESRD, is aggravated by the ischemia-reperfusion occurring with the grafting. Long-term immunosuppressant treatment causes hypertension and renal damage and further exacerbates it. Factors, as ET-1, ADMA, and FGF23, are involved in the impaired vasorelaxation and the renal damage of transplanted patients (see [3]).

Cyclosporine, a potent stimulus for ET-1 production and an inhibitor of the L-arginine /nitric oxide (NO) pathway, contributes by these mechanisms to post-transplant hypertension. Moreover, ET_A receptor expression increases in renal allografts and ERA treatment effectively suppresses fibrotic and proliferative responses in several allografts. Accordingly, selective ET_A, but not mixed ET_A/ET_B blockade, largely prevents chronic rejection and renal allograft injury, even in the absence of continued immunosuppression through mechanisms not improved by ARB treatment, suggesting that ERA have pronounced and independent immunomodulatory effects in the transplant recipients. However, available randomized clinical trials (RCTs) are too small to support recommendation of a specific class of drugs for treatment of transplant-associated hypertension (for a review see [3]).

Angiogenesis antagonists

A large number of agents, as anti-VEGF antibodies and small, orally active receptor tyrosine kinase inhibitors that block the VEGF signaling pathway, have been introduced to blunt angiogenesis occurring through endothelial cell proliferation (see [3]). VEGF causes tumor growth and metastatic spread via angiogenesis, but also promotes capillary repair in damaged glomeruli. Common adverse effects of angiogenesis antagonists are hypertension and kidney injury, which resemble preeclampsia, where the release of sFlt-1 in the bloodstream, by sequestering VEGF and placenta growth factor, is held to produce an antiangiogenic state. As in preeclampsia, activation of the ET system occurs in cancer patients treated with the receptor tyrosine kinase inhibitors sunitinib and regorafenib. Thus, inhibition of angiogenesis leads to ET-1 activation, particularly when NO bioactivity is blunted.

Hyperhomocysteinemia

Hyperhomocysteinemia usually derives from a gene-environment interaction involving a low folate intake and the methylene-tetra-hydro-folatereductase (MTHFR) gene (ID 4524) variant, particularly in elderly people and in the presence of reduced eGFR and left ventricular ejection fraction.. It detrimentally affects the endothelium via oxidative stress, with ensuing decreased NO production and bioactivity, and accumulation of the endogenous NO synthase inhibitor ADMA (see [3]).

As the kidney is the major site of homocysteine metabolism, unsurprisingly ESRD patients were found to develop hyperhomocysteinemia, which can be aggravated by the loss of B vitamins associated with dialysis. A recent meta-analysis failed to show a decrease in CV events and/or death, suggesting that homocysteine-lowering therapies with folic acid or vitamins B should not be used for CV risk reduction in ESRD patients [9]. However, there is a linear relationship between plasma homocysteine and CV risk events over the entire range of plasma homocysteine values. Accordingly, a benefit from homocysteine lowering may conceivably be expected only in those with overt hyperhomocysteinemia before they develop ESRD and not in the population at large, wherein the beneficial effect seen in a subset of the subjects can be markedly diluted, or in the patients with with full-blown ESRD where benefits, even if present, can hardly be seen.

Endothelial factors and kidney protection

Whether specifically targeting endothelial dysfunction may ultimately improve CV and renal outcomes in hypertension and CKD patients remains to be demonstrated in adequately designed, long-term RCTs. A holistic approach aimed at treating hypertension, hyperlipidemia, and diabetes mellitus (with/without diabetic nephropathy) should be the optimal strategy to prevent or retard CKD.

In CKD patients use of mineralocorticoid receptor antagonists, especially if combined with ACE inhibitors and/or ARBs, significantly decreased microalbuminuria (see [3]). However, caution should be exercised in prescribing these agents to CKD patients because of the risk of hyperkalemia. Serial measurement of serum K^+ levels is mandatory in these patients if the mineralocorticoid antagonists are held to be life-saving.

Studies performed to target the molecular pathways involved in endothelial dysfunction in CKD and hypertension included the antioxidant N-acetylcysteine, the phosphodiesterase type 5 (PDE5) inhibitors sildenafil and vardenafil, and the phosphodiesterase inhibitor with antiplatelet/antithrombotic effect cilostazol (see [3]). These drugs effectively prevented glomerular hypertension and hyperfiltration in animal models, but the evidences in humans derive only from small-sized short-term RCTs.

Conclusions and recommendations

Translation of the new overwhelming knowledge on the role of endothelium-related mechanisms into clinical practice has been slow with current pharmacologic tools, likely because of the difficulty of disentangling the relative role of each putative pathogenic factor. Hence, a challenge for the next decade is to undertake further specific research by designing more RCTs [6] focused at testing strategies for preserving endothelial function and GFR and treating renal fibrosis and reducing end-stage renal disease.

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