Endothelium-derived ET-1 and the development of renal injury

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ENDOTHELIN-1 (ET-1) is an endogenous 21 amino acid peptide that has powerful vasoactive properties. ET-1 is produced by many cell types including endothelial cells (40), cardiomyocytes (36), mesangial cells (31), and different segments of the nephron, especially collecting ducts (22). Increased activity of the ET-1 system has been described in several cardiovascular and renal diseases (25, 28). ET-1 stimulates two G protein-coupled receptor subtypes, ETA and ETB receptors, with the same affinity for both receptors (7). Activation of each receptor subtype leads to different, and often opposite, physiological and pathophysiological results (3). Renal cortical and inner medullary tubules are rich in ETB receptors, whereas outer medullary tubules express both ETA and ETB receptors. Over-activation of the renal ETA pathway leads to hypertrophy, inflammation, and fibrosis. Actions of the ETB pathway promote clearance of ET-1 from circulation, stimulation of nitric oxide, and/or prostacyclin release, as well as increased sodium and water excretion (23). Several cell-type-specific endothelin pathway knockout mouse models and an ETB receptor-deficient rat model facilitate in-depth investigations into the activation of cellular source(s) and actions of ET-1. This review highlights the rationale for the use of genetic rodent models to elucidate the autocrine and/or paracrine mechanisms of ET-1-dependent development of renal apoptosis and injury. Based on the literature and our own preliminary findings, studying tunicamycin-induced renal apoptosis in two ET-1 genetic rodent models provides rationale for a working hypothesis that endothelium-derived ET-1 induces renal tubular apoptosis by a paracrine mechanism.

Renal injury is preceded by tubular apoptosis and loss of nephrons (26). Apoptotic cell death is characterized by a series of changes in cellular morphology, such as shrinkage of the cell membrane, condensation of nuclear chromatin, cellular fragmentation, and engulfment of the apoptotic bodies by neighboring cells (21). Different vasoactive peptides have been implicated in the regulation of cellular apoptosis; however, contradictory reports in the literature do not provide a clear role of ET-1 in apoptosis and renal injury. Some studies indicate that ET-1 attenuates apoptosis in vascular smooth muscle cells (39), endothelial cells (11), and fibroblasts (33), whereas others describe pro-apoptotic effects of ET-1 in vascular smooth cells (5) or different areas of the kidney such as glomeruli, tubular cells, and interstitial cells (17).

Part of the confusion regarding the role of the endothelin pathway in apoptosis may be related to the conditions under study and the ET receptors involved. Several publications report that ETA receptor activation promotes cell proliferation and cell survival in a rat model of polycystic kidney disease (PKD) (16) during kidney development (41), in vascular smooth muscle cells (34), or in cardiomyocytes (27), whereas others describe pro-apoptotic effects of the ETA receptor in a model of chronic renovascular disease (19). Furthermore, ETB-selective agonists decreased apoptosis in rat endothelial cells (33) and ETB antagonists lead to increased apoptosis in rat and human endothelial cells (11, 32). Cancer studies show that activation of the ETB receptor is considered a survival mechanism (24), whereas the inhibition or loss of the ETB receptor is protective against apoptosis in renal tubular cells of a mouse model of PKD (6) or neurons subjected to hypoxia-ischemia (35). It is clear from all these reports that the ET pathway is involved and that a better understanding of the mechanisms by which ET-1 leads to renal injury, especially renal apoptosis, is needed. Thus utilizing a variety of genetic models would be...
advantageous for the study of ET-1-dependent mechanisms in renal apoptosis and injury.

**Genetic Rodent Models: ET-1/ET\textsubscript{A} Pathway Versus ET-1/ET\textsubscript{B} Pathway**

To better dissect the role of each of the components of the ET-1 system in the development of cardiovascular and renal disease, several genetic modifications have been performed in laboratory rodents (for review see Ref. 23). In 2010, a vascular endothelial cell ET-1 knockout mouse (VEET KO) was created (20). The VEET KO mouse has reduced plasma ET-1 concentrations and reduced ET-1 expression in vascular segments. Experiments using this model have shown that vascular endothelium-derived ET-1 mediates vascular inflammation and neointima formation in atherosclerosis (2), promotes cardiac fibrosis in diabetes (38), and stimulates glomerular formation of reactive oxygen species in response to high-salt diet or hypoxia (15).

Several genetic rodent models were generated to better understand the differential roles of the ET\textsubscript{A} and ET\textsubscript{B} receptors. For instance, rats without a functional ET\textsubscript{B} receptor display endothelial dysfunction (30) and salt-sensitive hypertension (12) via overactivation of ET\textsubscript{A} receptors (29). In addition, Kohan et al. demonstrated that collecting duct-specific knock-out of ET-1 or the ET\textsubscript{B} receptor, but not the ET\textsubscript{A} receptor, leads to hypertension, sodium retention (1), and decreased plasma vasopressin levels (13). These studies highlight the importance of the ET-1/ET\textsubscript{B} pathway in the collecting duct as a physiological regulator of ENaC activity (4, 14) and systemic blood pressure. Interestingly, specific vascular smooth muscle disruption of the ET\textsubscript{A} receptor in mice leads to reduced blood pressure responses to high-salt diet and reduced vascular reactivity, highlighting the important role of this receptor in mediating the ET-1-induced vasoconstriction (10). An important question is the degree to which the loss of ET\textsubscript{B} receptor function results in elevated ET\textsubscript{A}-dependent effects that are unopposed.

**Working Hypothesis: Endothelium-Derived ET-1 Mediates Renal Tubular Apoptosis**

ET-1 is released from the basolateral cellular side highlighting the role of this peptide as an autocrine/paracrine factor (37). Recently, Widyantoro et al. (38) reported that endothelium-derived ET-1 is necessary for the development of cardiac fibrosis during diabetes, further emphasizing the paracrine actions of ET-1 and its role in tissue damage. Preliminary evidence from our laboratory show that control mice treated overnight with tunicamycin have significant levels of apoptosis in the outer medullary tubules, but not in renal vessels (9). We found that the outer medullary tubules of VEET KO mice are protected against the development of apoptosis in response to tunicamycin (9), indicating a role for the pro-apoptotic effects of endothelium-derived ET-1 on kidney tubular cells in a paracrine manner. Moreover, given that the outer medulla expresses ET\textsubscript{A} receptors, these studies suggest that the ET\textsubscript{A} receptor is pro-apoptotic in this particular rodent model. Tunicamycin can induce endoplasmic reticulum (ER) stress- and mitochondrial-mediated apoptosis (18), although the direct involvement of ET-1 was not tested in this study. Using the ET\textsubscript{B}-deficient rat as an experimental model, our group previously reported that pharmacological blockade of the ET\textsubscript{A} receptor ameliorates tunicamycin-induced renal ER stress and apoptosis in transgenic control rats, while failing to do so in the ET\textsubscript{B}-deficient rats (8). These results highlight the critical role of ET\textsubscript{A} receptor activation in renal apoptosis as well as the protective effect of the ET\textsubscript{B} receptor against tunicamycin-induced renal apoptosis. Interestingly, acute treatment with tunicamycin did not alter circulating ET-1 levels. Based on our preliminary results and information from the literature, we propose the working hypothesis that activation of endothelium-derived ET-1 induces renal tubular apoptosis in a paracrine manner (Fig. 1).

**Perspectives and Significance**

To test our proposed hypothesis, our laboratory will utilize the VEET KO mouse model with pharmacological blockade of the ET\textsubscript{A} and ET\textsubscript{B} receptors to assess the effect on tunicamycin-induced renal apoptosis. We will further differentiate between the actions of renal or nonrenal sources of ET-1 in the development of tunicamycin-induced renal apoptosis with renal transplantation studies in flox control and VEET KO mice. The preliminary results and the proposed studies with genetic models will determine the potential therapeutic value of targeting the endothelial ET-1 system to prevent the development of renal injury and apoptosis.

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**DISCLOSURES**

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**AUTHOR CONTRIBUTIONS**

Author contributions: C.D.M. and J.S.P. conception and design of research; C.D.M. performed experiments; C.D.M. analyzed data; C.D.M., D.M.P., and J.S.P. interpreted results of experiments; C.D.M. prepared figures; C.D.M. and J.S.P. interpreted results of experiments; C.D.M. analyzed data; J.S.P. provided critical revisions of the manuscript; J.S.P. final approval of manuscript.

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![Fig. 1. Working hypothesis. Vascular endothelium-derived endothelin-1 (ET-1) mediates renal injury in cardiovascular disease by inducing tubular apoptosis.](image-url)
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