Endothelin and Tubulointerstitial Renal Disease

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Summary: All components of the endothelin (ET) system are present in renal tubular cells. In this review, we summarize current knowledge about ET and the most common tubular diseases: acute kidney injury (AKI) and polycystic kidney disease. AKI originally was called acute tubular necrosis, pointing to the most prominent morphologic findings. Similarly, cysts in polycystic kidney disease, and especially in autosomal-dominant polycystic kidney disease, are of tubular origin. Preclinical studies have indicated that the ET system and particularly ETα receptors are involved in the pathogenesis of ischemia-reperfusion injury, although these findings have not been translated to clinical studies. The ET system also has been implicated in radiocontrast-dye–induced AKI; however, ET-receptor blockade in a large human study was not successful. The ET system is activated in sepsis models of AKI; the effectiveness of ET blocking agents in preclinical studies is variable depending on the model and the ET-receptor antagonist used. Numerous studies have shown that the ET system plays an important role in the complex pathophysiology associated with cyst formation and disease progression in polycystic kidney disease. However, results from selective targeting of ET-receptor subtypes in animal models of polycystic kidney disease have proved disappointing and do not support clinical trials. These studies have shown that a critical balance between ETα and ETβ receptor action is necessary to maintain structure and function in the cystic kidney. In summary, ETs have been implicated in the pathogenesis of several renal tubulointerstitial diseases, however, experimental animal findings have not yet led to use of ET blockers in human beings.

Keywords: Endothelin, acute kidney injury, polycystic kidney disease, ADPKD, ET-1, ETα, ETβ

Acute kidney injury (AKI) is a clinical syndrome that is characterized by a rapid decrease in glomerular filtration rate (GFR). The decrease in renal function is accompanied by retention and accumulation of waste products such as urea and creatinine, and very often is characterized by decreased urine output, increased plasma potassium and phosphate concentrations, metabolic acidosis, and changes in body fluid balance. It can result from several disorders that affect the kidney acutely and many different pathways are involved in the complex pathogenesis. The cause of AKI differs somewhat depending on the population (community-acquired), country, and site of onset (hospital-acquired or before hospitalization). Renal ischemia-reperfusion injury (IRI) represents a very common cause of AKI: sepsis-induced AKI is the most common form of AKI observed in critically ill patients and accounts for at least 50% of cases in the intensive care unit. Contrast-induced nephropathy (CIN) has become the third leading cause for hospital-acquired AKI because interventional cardiac procedures are increasing steadily. Evidence is accumulating that the renal endothelin (ET) system is involved in the development and maintenance of AKI.

ENDOTHELIN IN ISCHEMIA-REPERFUSION INJURY OF THE KIDNEY

Shortly after the discovery of ET as a very potent vasoconstrictor, it was suggested that this peptide plays an important role in the pathogenesis of AKI. Infusion experiments with rat kidneys showed that exogenous ET reduces renal blood flow and GFR, resembling hypoperfusion and hypofiltration observed in postischemic kidneys. At the same time, it was shown that endogenous ET was increased in renal tissues in a rat model of ischemic acute renal failure. Wilhelm et al showed that renal ET-1 concentrations are increased during the initial 24 hours after reperfusion in experimental ischemic AKI. Firth and Ratcliffe reported long-lasting increases in renal ET-1 messenger RNA (mRNA) expression after ischemia, suggesting a role for ET in maintaining postischemic glomerular dysfunction. These and ensuing studies clearly showed that increased renal ET-1 mRNA expression and ET-1 formation occurs with renal IRI.

Subsequently, several experiments investigating the effects of selective and nonselective ET-receptor blockade in the setting of renal IRI were published. Although several investigators were able to show beneficial effects on renal function and morphology in animal models of IRI, there also were conflicting data showing no functional protection with ET-receptor blockade in postischemic renal injury.
suggested differences in the time point of drug treatment as a possible explanation (ie, before, during, or after the ischemic period). However, Wilhelm et al\textsuperscript{20} showed that treatment with the nonselective ET\textsubscript{A}/ET\textsubscript{B}-receptor antagonist tezosentan both before and after the ischemic period successfully decreased serum creatinine level, increased GFR, and maintained renal architecture in kidneys after ischemia. Recently, Zager et al\textsuperscript{19} investigated the effects of ET\textsubscript{A}- or ET\textsubscript{B}-specific antagonists in a mouse model of IRI both before and after injury. Over the experimental period of 2 weeks after unilateral ischemic injury, intrarenal ET-1 production increased as well as the expression of ET\textsubscript{A}, but not ET\textsubscript{B}. Although ET\textsubscript{A} blockade provided protective effects on the kidney, ET\textsubscript{B} blockade had no such effects. The investigators concluded that interaction between ET-1 and ET\textsubscript{A} might have an important role in AKI leading to chronic kidney disease. These results suggested that intervention with ET\textsubscript{A}-receptor antagonists could be a therapeutic option for slowing or preventing the progression from postischemic AKI to chronic kidney disease (CKD).\textsuperscript{18}

Comparable results were found in a different model of IRI in mice lacking ET-1 in vascular endothelial cells. One day after IRI induced by bilateral clamping of the renal pedicles, these animals showed significantly less vascular and tubular injury than the control group. In control wild-type mice, vascular and tubular injury were associated with up-regulated ET-1 and ET\textsubscript{A} expression.\textsuperscript{21} This experiment also underlined the importance of endothelial cells in the microvasculature in IRI. Endothelial dysfunction is a major contributor to ischemic renal damage and is characterized by an imbalance between the release of ET-1 and nitric oxide (NO).\textsuperscript{22} Damage to vascular endothelial cells during reperfusion may lead to the release of ET from injured endothelial cells, followed by sustained intrarenal vasoconstriction and thus hypoxic injury of the adjacent tubules.\textsuperscript{8,23}

In diabetes, these events may be even more pronounced because insulin resistance results in widespread endothelial dysfunction with increased levels of ET-1 and decreases in NO through disruption of NO synthase. This further impairs renal vascular autoregulation.\textsuperscript{22} In rats treated with streptozotocin, IRI led to a more severe renal phenotype than in nondiabetic animals. With the use of selective ET\textsubscript{A} blockers, a partial improvement of kidney function in diabetic rats that underwent IRI was observed.\textsuperscript{22} RNA interference with a short hairpin RNA for ET\textsubscript{A} partially silenced gene expression and led to improved renal function and structure in IRI injury in rats\textsuperscript{24} (Table 1).

**ENDOTHELIN IN CONTRAST DYE–INDUCED ACUTE KIDNEY INJURY**

Acute kidney injury frequently occurs after exposure to iodinated contrast media for cardiac angiography and coronary/vascular interventions. CIN is the third most common cause of new-onset renal injury in hospitalized patients and is associated with increased morbidity and mortality.\textsuperscript{25} Contrast media might exert direct tubular cell toxicity mediated by free radicals, oxidative stress, cytokine-induced inflammation, and apoptosis.\textsuperscript{25,26} Renal medullary hypoxia caused by a selective reduction in blood flow to the outer medulla may be an important contributory factor to the development of CIN\textsuperscript{27}; when oxygenation is impaired, medullary hypoxic injury with necrosis of the tubules occurs.\textsuperscript{28} Endothelin may be involved in several of these pathogenic processes. In animal experiments, intravenous radiocontrast infusion increases plasma and urinary ET-1 levels.\textsuperscript{29,30} Contrast media can directly damage endothelial cells in renal vessels,\textsuperscript{31} which may explain the observed increase in ET-1 levels. Administration of ET-receptor antagonists prevented renal vasoconstriction\textsuperscript{32,33} as well as improved functional and structural markers\textsuperscript{32–36} in animal models of radiocontrast nephrotoxicity (Table 2).

Results from studies of the human renal ET system in patients with CIN are scarce. In 1997, Clark et al\textsuperscript{37} showed in 19 patients undergoing arteriography that circulating ET-1 levels increased after large-volume, but not small-volume, contrast exposure. An even smaller study (n = 6) investigated the role of ET in CIN in patients with chronic renal failure. Application of contrast media increased urinary ET excretion (corrected by creatinine concentration) in patients with pre-existing impaired renal function but not in patients with normal renal function.\textsuperscript{38} A few other studies have investigated ET-1 plasma levels before and after application of contrast media and found either no differences\textsuperscript{39} or an increase in plasma ET levels after contrast administration.\textsuperscript{40,41} Ulas et al\textsuperscript{42} showed an increase in urinary and plasma ET-1 concentrations in 78 patients but provided no information on renal function. Until now, only two larger prospective clinical studies of the renal ET system before and after exposure to contrast media in patients with renal disease have been conducted. The first study showed that ET-receptor antagonism and intravenous hydration exacerbated radiocontrast nephrotoxicity compared with hydration alone in patients with chronic renal insufficiency undergoing cardiac angiography.\textsuperscript{43} However, the dose of the ET\textsubscript{A}/ET\textsubscript{B} antagonist was not established in phase 2 studies in advance of this phase 3 trial. A large observational study found a decrease in urinary ET concentration as well as the urinary ET-1/creatinine ratio after application of contrast medium in patients with diabetes or renal impairment undergoing coronary angiography.\textsuperscript{3} The urinary ET-1 concentration and the urinary ET-1/creatinine ratio after contrast application were higher in patients who had a persistent decrease in GFR of at least 25% after 90 days of follow-up evaluation. No major
long-term complications of CIN (need for dialysis, rehospitalization, or death) were associated with renal ET levels in this study.

The findings from these two larger studies in human beings, and especially the negative effects of ET-receptor blockade, are contradictory to promising results in CIN from animal studies. This discrepancy could be partially attributable to a higher incidence of hypotension in the treated population in the clinical trial. Further reasons are discussed extensively by Wang et al. Another point that has to be taken into account is that the existing animal models for CIN are probably not of adequate translational value. Therefore, we need better CIN animal models closer to human pathophysiology (models should consider risk factors leading to CIN such as diabetes or heart failure). Clinical development requires proper dose finding in phase 2 trials before initiating an outcome study to avoid unwanted effects on blood pressure (Table 3).

ENDOTHELIN IN SEPSIS-INDUCED ACUTE KIDNEY INJURY

Increased ET-1 plasma levels in septic patients are associated with the severity of the illness and may be involved in renal complications during sepsis. The ET-1 level in kidney tissue is increased in a time-dependent manner after lipopolysaccharide (LPS) administration to rats. Increased ET levels in plasma and tissues are believed to be a consequence of an up-regulation of ET-1 synthesis in vascular endothelial cells of various organs as well as endothelial injury. Over the past few years of research on AKI, a new concept for the pathogenesis of renal failure in early sepsis has emerged. It is now being questioned if global renal ischemia resulting from systemic hypotension is the main reason for the loss of GFR, a traditionally held dogma. It has been shown in animal models and in human studies.

<table>
<thead>
<tr>
<th>Reference</th>
<th>Antagonist/Knockout</th>
<th>Model Outcome</th>
<th>Model</th>
<th>Species</th>
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<tr>
<td>11</td>
<td>ETA (BQ-123)</td>
<td>Preserved renal function</td>
<td>Bilateral clamping of renal artery and vein</td>
<td>Rats</td>
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<tr>
<td>12</td>
<td>ETA (BQ-123)</td>
<td>Treatment before renal arterial clamping: beneficial effects on renal function</td>
<td>Bilateral renal artery clamp for 45 min</td>
<td>Rats</td>
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<tr>
<td>13</td>
<td>ETA (BQ-123)</td>
<td>Treatment only during the reperfusion period: no effects on renal function</td>
<td>Bilateral renal artery clamp for 45 min</td>
<td>Rats</td>
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<tr>
<td>15</td>
<td>ETA/ETB (SB 209670)</td>
<td>In rats with severe AKI: improved survival rate</td>
<td>Uninephrectomized + 45-min renal artery clamp</td>
<td>Rats</td>
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<td>17</td>
<td>ETA (SB 234551)</td>
<td>Reduced apoptosis</td>
<td>Uninephrectomized + 45-min renal artery clamp</td>
<td>Rats</td>
</tr>
<tr>
<td>18</td>
<td>ETA (Atrasentan)</td>
<td>Decreased tubule/microvascular injury, total preservation of renal mass</td>
<td>Uninephrectomized + 30-min renal artery clamp</td>
<td>CD-1 mice</td>
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Abbreviation: ARF, acute renal failure.

ENDOTHELIN IN SEPSIS-INDUCED ACUTE KIDNEY INJURY

Increased ET-1 plasma levels in septic patients are associated with the severity of the illness and may be involved in renal complications during sepsis. The ET-1 level in kidney tissue is increased in a time-dependent manner after lipopolysaccharide (LPS) administration to rats. Increased ET levels in plasma and tissues are believed to be a consequence of an up-regulation of ET-1 synthesis in vascular endothelial cells of various organs as well as endothelial injury. Over the past few years of research on AKI, a new concept for the pathogenesis of renal failure in early sepsis has emerged. It is now being questioned if global renal ischemia resulting from systemic hypotension is the main reason for the loss of GFR, a traditionally held dogma. It has been shown in animal models and in human studies.
that sepsis-induced AKI may occur despite an increase or no decrease in total renal blood flow (RBF). Changes in the relation of afferent-efferent arteriolar resistance, which are triggered by intrarenal hemodynamic factors, including ET-1, might modulate GFR. The role of ET-1 in renal complications in sepsis has been investigated in animal experiments using ET-receptor antagonists. ET-receptor blockade is useful in preventing albuminuria during endotoxin shock in conscious, chronically catheterized rats. Mitaka et al. showed that non-selective ET-receptor blockade improved LPS-induced decreases in urine volume, RBF, creatinine clearance, and urine osmolality in dogs. Improved renal function also was observed with dual ET<sub>A</sub>/ET<sub>B</sub> blockade in neonatal piglets during endotoxemia. In contrast, neither selective nor combined ET<sub>A</sub> and ET<sub>B</sub> blockade improved GFR in a rat model of endotoxin-induced acute kidney dysfunction. Selective ET<sub>B</sub> antagonism caused renal vasoconstriction and reduced RBF in this experimental setting. According to the investigators, this showed that ET<sub>B</sub> might be involved in the preservation of renal blood flow via renal vasodilation. In this experiment, no change in medullary circulation was observed with ETA blockade, which is in contrast to the findings in endotoxemic pigs, suggesting that ET reduces renal medullary perfusion.

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<th>Table 2. ET-Receptor Blockers in Animal Models of CIN</th>
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Abbreviation: L-NAME, L-NG-nitroarginine methyl ester.

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<th>Table 3. Human Studies of CIN</th>
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Abbreviation: CT, computed tomography.
owing to activation of ET<sub>A</sub>.<sup>53</sup> A possible explanation may be that medullary microvascular flow was not reduced by LPS in the first mentioned study.<sup>59</sup> Fenhhammer et al<sup>60</sup> previously showed in a similar model that treatment with tezosentan, a dual ET<sub>A</sub>/ET<sub>B</sub> antagonist, improved renal artery blood flow and renal cortical microcirculation in endotoxemia, but had no effect on urine production. Therefore, the investigators hypothesized that selective ET<sub>A</sub> antagonism would improve diuresis by preserving ET<sub>B</sub> function. A further experiment in endotoxemic pigs did not confirm this because treatment with an ET<sub>A</sub> antagonist had no significant effects on diuresis or creatinine clearance. However, ET<sub>A</sub> antagonism attenuated endotoxemia-induced microcirculatory impairment and ischemia in the renal medulla. These findings question the advantages of a selective ET<sub>A</sub> antagonism compared with combined ET<sub>A</sub>/ET<sub>B</sub> antagonism. Because LPS induces expression of both renal receptors,<sup>48</sup> dual ET-receptor blockade may be promising in preventing renal dysfunction in sepsis. However, the individual functional contributions of ET and its receptors in sepsis-induced AKI need to be investigated further. Most likely, they are important components of the pathogenesis of sepsis-induced AKI, but their exact role in the complex glomerular and periglomerular changes in hemodynamics and microcirculatory blood flow in the kidney is not clear.<sup>50</sup> Discrepancies and contradictory results in the aforementioned studies are obvious and could relate to differences in the animal models of sepsis. Varying observation periods, diverse pharmacologic substances, and, most importantly, species differences in the distribution of ET receptors in the kidney, might have functional implications. Therefore, further research is needed to better understand the role of the ET system in sepsis-induced AKI (an overview of the preclinical studies is shown in Table 4).

### POLYCYSTIC KIDNEY DISEASE: BACKGROUND

Autosomal-dominant polycystic kidney disease (ADPKD) is the most common genetic kidney disease in human beings with an estimated prevalence of 1:400 and 1:1,000. It is the fourth most common cause of end-stage renal disease (ESRD), which occurs in 50% of affected individuals before the age of 60 years.<sup>51</sup> Almost all cases of ADPKD are caused by germline mutations in <i>PKD1</i> or <i>PKD2</i>. The ADPKD proteins polycystin-1 (460 kDa) and polycystin-2 (110 kDa) play critical roles in maintaining normal tubular structure and function through the regulation of multiple signaling pathways involving Ca<sup>2+</sup>, mechanistic target of rapamycin (mTOR), and cyclic adenosine monophosphate homeostasis.<sup>62,63</sup>

Clear genotype-phenotype correlations have been reported in ADPKD.<sup>64</sup> Nonetheless, it is well known that there is marked intrafamilial phenotypic variability of ESRD onset in many PKD1 or PKD2 pedigrees.<sup>65</sup> Conversely, there is concordance for ESRD between identical twins compared with their siblings.<sup>66</sup> These studies confirm the likely importance of nonallelic genetic factors (other genes, epigenetic modification) or gene-environment interactions in determining individual phenotype.

The pathophysiology of ADPKD is complex and includes changes in cell proliferation, apoptosis, fluid secretion, extracellular matrix formation, apoptosis,
angiogenesis, defective planar cell polarity, and cilia-mediated mechanosensation. The molecules involved in these processes could play rate-limiting steps in renal disease progression. Cysts commonly occur in other organs and especially in the liver and pancreas; major noncystic manifestations include hypertension (70%), left ventricular hypertrophy, aortic aneurysms, and cardiac valvular abnormalities, indicating that ADPKD is more than a kidney disease.

THE ROLE OF ENDOTHELINS

ET-1 has been reported to exert multiple effects on different aspects of renal physiology. Apart from circulating ET-1, there is good evidence that many renal cell types, including tubular cells, synthesize and bind ET-1. As discussed earlier, two major ET-receptor subtypes, ETA and ETB, have been shown to mediate ET-1 action in the kidney, but often with opposing effects. The potential role for endothelins as modifying factors in ADPKD disease progression have been studied by several groups, are discussed later, and are summarized in Tables 5 and 6.

SINGLE-NUCLEOTIDE POLYMORPHISMS OF ET-1 AND ETA IN HUMAN ADPKD DISEASE PROGRESSION

Single-nucleotide polymorphisms (SNPs) of ET-1 and ETA genes have been shown to have a modest effect on the age of ESRD onset in ADPKD. In one study investigating the influence of an SNP ET\textsubscript{A} (C/T polymorphism in exon 8) on disease progression in 193 ADPKD patients (87 males, 106 females), females with the CC genotype reached ESRD at a significantly later age than CT heterozygotes (CC, 57.4 ± 8.1 y; CT, 53.0 ± 9.1 y; and TT, 54.5 ± 6.4 y). This SNP was also associated with a lower pulse pressure and thus could be protective in ADPKD patients. The same group also examined the influence of three SNPs in ET-1 (K198N, 3A/4A, and T-1370G) on progression to ESRD in 205 ADPKD patients. Of interest, carriers of the 4A allele in combination with the 198N allele (4A/4A, 3A/4A + 198KN,NN) had a significantly lower age of ESRD onset (47.1 ± 8.7 y), 6 years younger than the carriers of other genotypes. The deleterious effect of the combination of 4A and 198N alleles of SNPs of ET-1 could be owing to a higher processing rate of preproET into ET-1 in carriers of the 198N allele, leading to the higher ET-1 plasma levels. These studies suggest that activation of the systemic ET-1 system in human ADPKD could participate in disease progression, possibly by increasing systemic blood pressure and/or reducing RBF.

PLASMA OR CYST ET-1 LEVELS IN HUMAN ADPKD

Many renal cell types can synthesize and bind ET-1, indicating its potential as an autocrine or paracrine factor. In one study, concentrations of immunoreactive ET-1, cyclic adenosine monophosphate, and epidermal growth factor were found to be increased in human ADPKD cyst fluid. Another study reported that patients

| Table 5. Preclinical Studies Linking the ET System With Polycystic Kidney Disease |
|--------------------------------------|------|---------------------------------|-----------------|
| Reference | Antagonist | Outcome | Model | Species |
| 82 | Develop renal cysts but not hypertension | Blood pressure–independent model of ET\textsubscript{1}-induced renal pathology | hET\textsubscript{1} transgenic mice |
| 81 | Renal cysts apparent prior to glomerulosclerosis, interstitial fibrosis, and loss of GFR at 14 months | Renal cysts apparent by 3 months Salt-sensitive hypertension | mET\textsubscript{1} transgenic mice |
| 80 | ETA (LU 135252) | Tubular proliferation Cyst growth | ADPKD | Han:Sprague-Dawley rats |
| ETA/ETB (LU 224332) | Additional blockade of the ET\textsubscript{1}-receptor attenuated these effects | ADPKD | Pkd2 (WS25/-) mice |
| 86 | ETB (A-192621) | Accelerated cystic kidney disease | ADPKD |
| ETA (ABT-627) | Abolished effects of ET\textsubscript{B} blockade when given simultaneously | | |
| 83 | ETB (BQ-3020) | Hypoxia-induced tubular ET-1 synthesis Stimulated endogenous ET-1 synthesis with BQ-3020 | In vitro primary cultured cell system Human kidney cells |

Abbreviations: hET\textsubscript{1}, human ET-1; mET\textsubscript{1}, mouse ET-1
with ADPKD (hypertensive and normotensive) had increased plasma levels of ET-1 in comparison with patients with essential hypertension or healthy subjects. These studies suggested that both circulating and local ET-1 systems are activated abnormally in human ADPKD.

**ET-1 AND ET-RECEPTOR EXPRESSION IN PKD**

Increased levels of ET-1 have been reported in cystic kidneys from experimental models and human disease, although there are notable differences in the expression of ET-receptor subtypes. In the congenital polycystic kidney (cpk) mouse model of PKD, renal ET-1 mRNA levels were 3.2-fold greater than controls at 1 week, and 10-fold greater at 3 weeks when cystic disease was at its height. Similarly, renal ETA and ETB mRNA levels increased 4.2- and 6.3-fold over control kidneys, respectively. In the Han:SPRD rat model of PKD, significantly increased tissue levels of ET-1 were found in cystic kidneys, but ETA and ETB density were reported to be decreased markedly. In contrast, a study in human ADPKD cystic kidneys reported that ETA mRNA levels were 5- to 10-fold higher than non-ADPKD controls, whereas ETB mRNA was unchanged. In total kidney ETA mRNA levels in human ADPKD was confirmed in two subsequent studies using microarray platforms.

Activation of the renal ET system in ADPKD could result from stimulation by changes in the circulating and local renin-angiotensin-aldosterone system owing to cyst enlargement, resulting in focal ischemia and tissue hypoxia, both of which are strong stimuli for ET-1 synthesis.

**ET-1 AND CYST INITIATION IN TRANSGENIC MICE**

Overexpression of ET-1 (human or mouse) in transgenic mice is sufficient to trigger cyst initiation. ET-1 transgenic mice developed multiple cortical kidney cysts at as early as 3 months of age in addition to interstitial fibrosis and glomerulosclerosis, thus providing direct evidence that ET-1 alone can contribute to the initiation and progression of cystic kidney diseases. Overexpression of ET-1 in these mice was not associated with systemic hypertension, although salt-dependent hypertension could be induced in older mice. The mechanism of cyst formation in these models mice was not clarified, although intrarenal ischemia and tubular occlusion were proposed as potential explanations.

**ET-1 AND TUBULAR CELL PROLIFERATION**

ET-1 was shown to be a potent mitogen in vitro for normal human renal tubular cells and interstitial fibroblasts; in addition, it can stimulate collagen I gene expression in fibroblasts. Tubular ET-1 synthesis has been documented in experiments from different species. Tubular cell proliferation is an early feature of precystic tubules in human ADPKD and many rodent PKD models. A role for ETB in mediating the mitogenic effect of ET-1 has been shown in human proximal tubular cells as part of a hypoxia-inducible, forward-feedback, autocrine loop involved in renal tubular regeneration.

**SELECTIVE ETA OR ETB BLOCKADE IN PKD RODENT MODELS**

To date, there have only been two studies using selective ETA or ETB antagonists to delay cystic disease progression in rodent PKD models. However, there was a notable difference in the ET-receptor subtype responsible for disease progression between the two models.

Chronic administration of an ETA-selective antagonist (LU135252 or darusentan) for 4 months led to more severe cystic disease in the Han:SPRD rat. This was associated with an increase in tubular cell proliferation. The deleterious effect of ETA blockade was partially reduced by a nonselective ET-receptor antagonist (LU224332), presumably by blocking unopposed ETB. Alternatively, ETA blockade indirectly could result in tubular cell proliferation through ischemia-induced hypoxia inducible factor–dependent tubular ET-1 synthesis and an ETB-mediated positive feedback loop. The finding that ETA blockade induces tubular cell proliferation is different from that reported in other...
models of CKD or prostate cancer and appears characteristic of PKD.

In a second study, Pkd2<sup>WS25/-</sup> mice were treated from 5 to 16 weeks of age with the highly selective, orally active, receptor antagonists atrasentan (or ABT-627, ETA) or A-192621 (ETB), singly or in combination. Selective ETA blockade also resulted in a significant increase in tubular cell proliferation in this model but, unlike the previous study, did not alter the overall cystic phenotype. Conversely, selective ETB blockade led to accelerated kidney disease (including interstitial inflammation and fibrosis) without altering tubular cell proliferation. The deleterious effects of ETB blockade were mediated through unopposed ETA action because these changes were normalized by combined ETA and ETB blockade. In addition, ETB blockade resulted in a decrease in urine volume, reduced urinary sodium excretion, increased urine osmolality, and renal cyclic adenosine monophosphate concentrations, features that suggest an enhancement of vasopressin action. It is likely, however, that vasopressin-independent pathways also are involved.

These results could be explained by recent studies in segment-specific knockout mice that have shown a physiological role for local ET-1 acting via ETB in regulating sodium and water excretion by kidney collecting ducts. ETB activation inhibits vasopressin action, thereby promoting diuresis and natriuresis. ETB blockade therefore would potentiate vasopressin action in cysts derived from the collecting duct and thus stimulate cyst expansion. A primary role for vasopressin in ADPKD disease progression likely is based on the efficacy of vasopressin V2-receptor antagonists in suppressing cyst growth in rodent models of Autosomal recessive polycystic kidney (ARPKD), ADPKD, and nephronophthisis, and in human beings.

**CLINICAL IMPLICATIONS**

Figure 1 summarizes the potential pathogenic mechanisms by which ET-1 could promote cyst growth in ADPKD. In rodent PKD models, the balance between ETA and ETB signaling appears to be critical for maintaining kidney structure and function. ETB activation may suppress vasopressin action and increase vasodilatation, tubular proliferation, and salt and ET-1 excretion in PKD, whereas ETA activation may increase vasoconstriction, vasopressin action, inflammation, and fibrosis. In human ADPKD kidneys, the increase in ETA expression observed in several studies could represent a compensatory rather than a deleterious response. Selective ETA blockade has been found to be effective in reducing proteinuria in CKD patients but has not been tested in ADPKD patients. Nonetheless, ETA blockade in the Pkd2<sup>WS25/-</sup> model did not alter cystic disease severity despite the increase in ETB-mediated tubular cell proliferation. This observation implies that an increase in cell proliferation alone does not stimulate cyst growth, presumably owing to a compensatory increase in apoptosis. Nonetheless, this could represent a potential longer-term safety signal for renal cancer. Selective ETB blockade apparently is deleterious and should not be used in ADPKD.

Numerous studies have shown that the ET system plays important roles in the complex pathophysiology associated with cyst formation and disease progression in ADPKD. However, results from selective targeting of both ET-receptor subtypes in animal models of PKD have proved disappointing and do not support further extension into clinical trials. These studies, however, have shown that a critical balance between ETA and ETB action is necessary to maintain kidney structure and function in the cystic kidney, especially through regulating the action of vasopressin. These results confirm that ET-1 and its receptors act as major modifying genes for ADPKD. Future challenges will be to translate these findings to alter disease severity or predict prognosis in human beings.

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