Physiology and pathology of endothelin-1 in renal mesangium

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Sorokin, Andrey, and Donald E. Kohan. Physiology and pathology of endothelin-1 in renal mesangium. Am J Physiol Renal Physiol 285: F579–F589, 2003; 10.1152/ajprenal.00019.2003.—Mesangial cells (MCs) play a central role in the physiology and pathophysiology of endothelin-1 (ET-1) in the kidney. MCs release ET-1 in response to a variety of factors, many of which are elevated in glomerular injury. MCs also express ET receptors, activation of which leads to a complex signaling cascade with resultant stimulation of MC hypertrophy, proliferation, contraction, and extracellular matrix accumulation. MC ET-1 interacts with other important regulatory factors, including arachidonate metabolites, nitric oxide, and angiotensin II. Excessive stimulation of ET-1 production by, and activity in, MC is likely of pathogenic importance in glomerular damage in the setting of diabetes, hypertension, and glomerulonephritis. The recent introduction of ET antagonists, and possibly ET-converting enzyme inhibitors, into the clinical arena establishes the potential for new therapies for those diseases characterized by increased MC ET-1 actions. This review will examine our present understanding of how ET-1 is involved in mesangial function in health and disease. In addition, we will discuss the status of clinical trials using ET antagonists, which have only been conducted in nonrenal disease, as a background for advocating their use in diseases characterized by excessive MC-derived ET-1.

mesangial cell; cell signaling; receptor; pathophysiology

REGULATION OF ENDOTHELIN-1 GENE EXPRESSION IN MESANGIAL CELLS

While there are three members of the endothelin (ET) family (ET-1, ET-2, and ET-3), ET-1 is the major renal isoform produced by and acting on the mesangial cell (MC). ET-1 mRNA encodes a 212-amino acid prepropeptide that is cleaved to 38-amino acid big ET-1, which, in turn, is converted by ET-converting enzymes (ECEs) to mature 21-amino acid ET-1 (116). There are seven ECE isoforms and three ECE genes: ECE-1a, -1b, -1c, -1d, -2a, -2b, and -3. ECE-1 and ECE-2 prefer big ET-1, whereas ECE-1 prefers ET-3 (50). The combination of a short ET-1 mRNA half-life (~15 min) (44) and limited intracellular storage of ET-1 results in a close parallel between mRNA levels and peptide secretion. Thus the release of active ET-1 peptide must be controlled via 1) regulation of gene transcription; 2) mRNA stabilization; and/or 3) regulation of ECE activity.

Present data primarily implicate transcriptional control of ET-1 synthesis. ET-1 mRNA stability is un-changed by thrombin or cytokines (25, 65), and limited data are available on the regulation of ECE expression or activity (21). Numerous stimuli modulate MC ET-1 gene transcription, including vasoactive substances, growth factors, cytokines, G protein-coupled receptor agonists, and oxygen radicals (Table 1). The cooperation of tissue-specific transcription factors conveys a degree of tissue-selective ET-1 mRNA transcription and ensures that ET-1 is not inappropriately activated (115). This cooperation is made possible by the presence of multiple regulatory elements in the ET-1 promoter, including binding sites for activator protein-1, GATA-2, CAAT-binding nuclear factor-1 (NF-1), and cell-specific transcription factors upstream of classic CAAT and TATAA boxes (93). Importantly, these regulatory elements operate in different cell types, promoting cell-specific regulation of ET-1 mRNA synthesis [e.g., endothelium-specific transcription factors Vezf1/DB1 (1) and cardiac-specific GATA-4 (72)].

Regulation of ET-1 production has been extensively investigated in MCs. The 5’-flanking region of the ET-1
gene encompasses positive regulatory elements (e.g., engaged by thrombin), whereas negative modulation is exerted by the distal 5’ portion (25). Upregulation of prepro-ET-1 expression requires p38 MAPK and PKC (Fig. 1). Thrombin and cytokines (TNF and IL-1) synergistically increase ET-1 expression in MCs, an effect requiring activation of p38 MAPK and PKC, whereas ERK, JNK/SAPK, or intracellular Ca\(^{2+}\) release is uninvolved (25). The events upstream of p38 MAPK activation likely involve TGF-β-activated kinase 1-binding protein-1, TNF receptor-associated factor 2, and several MEKKs (Fig. 1). The ET-1 promoter is also activated by phorbol myristate acetic acid or ectopic expression of PKC-β1 in MC (32).

In summary, MC ET-1 release is under complex regulation, with vasoconstrictor, profibrotic, inflammatory, and proliferative agents augmenting its release, whereas vasorelaxant agents tend to inhibit its production (Table 1). Because MC ET-1 production is essentially regulated at the transcriptional level, changes in its release and subsequent actions are relevant to sustained biological effects rather than short-term and rapid modulation of glomerular structure and function.

## ET-1 SIGNALING AND ACTIONS IN MCS

ET-1 activates a variety of signaling systems in MCs to effect alterations in cell contraction, hypertrophy, proliferation, and extracellular matrix accumulation (Table 2). These actions are subsequent to ET-1 binding to its heterotrimeric G protein-coupled receptors, ET receptor A (ETRA) and B (ETRB), both of which are expressed by MCs (32, 80, 103, 118). ET-1 binds to both receptor subtypes with high affinity (K\(_d\) in the 100 pM range) and typically exerts prolonged effects (up to several hours). Normal plasma levels of ET-1 average 1–2 pM, indicating that the peptide primarily functions as an autocrine or paracrine factor. This underscores the importance of viewing MC ET-1 action in the context of the glomerular microenvironment.

ET-1 receptors couple to members of the Gi, Gq, Gs, and G\(_{12/13}\) G protein families (19, 43, 56) with resultant modulation of a variety of signaling cascades, including cyclooxygenases, cytochrome P-450, nitric oxide synthases (NOS), the nuclear helix-loop-helix protein p8 (30), serine/threonine kinases, and tyrosine kinases (Fig. 2). Common to induction of many of these

![Fig. 1. Proposed signaling pathways regulating endothelin-1 (ET-1) mRNA transcription in mesangial cells. Upregulation of ET-1 expression requires p38 MAPK and PKC. TGF-β-activated kinase 1 (TAK1)-binding protein 1 (TAB1) is an essential cofactor required for activation of TAK1 by TGF-β and IL-1β. TAK1 recruits p38 MAPK via activation of MEK3 and MEK6. MEKK1 can also be stimulated by TNF in a TNF receptor-associated factor 2 (TRAF2)-dependent manner. Little is known about the regulation of MEKK2 and MEKK3. The ability of thrombin to activate MEKK2/3 is hypothetical. PKC is upregulated by diacylglycerol (DG) produced by phospholipase C (PLC) from phosphatidylinositol biphosphate (PIP2). ASK1, apoptosis signal-regulating kinase 1.](http://ajprenal.physiology.org/)

<table>
<thead>
<tr>
<th>Increase ET-1</th>
<th>Ref. No(s.)</th>
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<tbody>
<tr>
<td>Transforming growth factor-β(_1)</td>
<td>32, 121</td>
</tr>
<tr>
<td>Oxidized LDL</td>
<td>104</td>
</tr>
<tr>
<td>Reactive oxygen species</td>
<td>39</td>
</tr>
<tr>
<td>Endothelin-1</td>
<td>48</td>
</tr>
<tr>
<td>Cyclosporin A</td>
<td>62</td>
</tr>
<tr>
<td>PK-506</td>
<td>29</td>
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<tr>
<td>Hyperglycemia</td>
<td>31</td>
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<tr>
<td>Angiotensin II</td>
<td>42, 59</td>
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<tr>
<td>Vasopressin</td>
<td>42, 88</td>
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<tr>
<td>Thrombin</td>
<td>42, 88, 121</td>
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<tr>
<td>Platelet-derived growth factor</td>
<td>42, 88</td>
</tr>
<tr>
<td>Tumor necrosis factor</td>
<td>58</td>
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<tr>
<td>Interleukin-1</td>
<td>25</td>
</tr>
<tr>
<td>Phorbol ester</td>
<td>32</td>
</tr>
<tr>
<td>Insulin</td>
<td>5</td>
</tr>
<tr>
<td>Thromboxane A(_2)</td>
<td>121</td>
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<table>
<thead>
<tr>
<th>Decrease ET-1</th>
<th>Ref. No(s.)</th>
</tr>
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<tbody>
<tr>
<td>Atrial natriuretic peptide</td>
<td>62</td>
</tr>
<tr>
<td>Brain natriuretic peptide</td>
<td>62</td>
</tr>
<tr>
<td>Heparin</td>
<td>61</td>
</tr>
<tr>
<td>Angiotensin receptor blocker</td>
<td>4</td>
</tr>
<tr>
<td>Angiotensin-converting enzyme inhibitor</td>
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</tr>
<tr>
<td>cGMP</td>
<td>60</td>
</tr>
<tr>
<td>cAMP</td>
<td>88</td>
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ET-1, endothelin-1.
pathways in MCs, ET-1 activates PLC (49, 97, 98) and PKC (94). Increased inositol triphosphate levels are associated with cell alkalinization via augmented Na/H exchange and increased intracellular Ca^{2+} concentration ([Ca^{2+}]_i) (3, 94, 96, 97). The increase in [Ca^{2+}]_i is due to release from intracellular stores as well as influx from dihydropyridine-insensitive pathways (94). Lower ET-1 concentrations (0.1–10 pM) cause slow, sustained increases in [Ca^{2+}]_i that are dependent on Ca^{2+} influx through a voltage channel-independent mechanism, whereas higher ET-1 concentrations (≥100 pM) cause a rapid and transient increase that depends on Ca^{2+} release from intracellular stores via activation of PLC and PKC (97).

Hypertrophy and Proliferation

There is abundant evidence that ET-1 directly and indirectly (e.g., via PDGF) (49) stimulates MC mitogenesis (5, 28, 32, 80, 98) as well as partially mediating the proliferative response to other growth factors (such as TGF-β) (32, 80). The proliferative and hypertrophic responses to ET-1 are mediated by PKC-β and PKC-ε (94), which are activated by ET-1 via the Gi and Gq subtypes of G-protein-coupled receptors (49). PKC activation leads to the phosphorylation of Shc proteins, which are involved in the activation of the Ras/MAPK signaling pathway, leading to cell proliferation and hypertrophy (94, 97).

Table 2. Stimulatory or inhibitory effects of ET-1 on substances released by mesangial cells

<table>
<thead>
<tr>
<th>Stimulated Substances</th>
<th>Inhibited Substances</th>
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<tbody>
<tr>
<td>Endothelin-1</td>
<td>Matrix metalloprotease-2</td>
</tr>
<tr>
<td>Tumor necrosis factor</td>
<td>Cytokine-stimulated NO</td>
</tr>
<tr>
<td>Platelet-derived growth factor</td>
<td>NO, nitric oxide.</td>
</tr>
<tr>
<td>Prostaglandin E_{2}</td>
<td>Matrix metalloprotease-2</td>
</tr>
<tr>
<td>Thromboxane B_{2} (mild)</td>
<td>Cytokine-stimulated NO</td>
</tr>
<tr>
<td>Plasminogen activator inhibitor</td>
<td>NO, nitric oxide.</td>
</tr>
<tr>
<td>Fibronecctin</td>
<td>Matrix metalloprotease-2</td>
</tr>
<tr>
<td>Type IV collagen</td>
<td>Cytokine-stimulated NO</td>
</tr>
<tr>
<td>Type I collagen</td>
<td>NO, nitric oxide.</td>
</tr>
<tr>
<td>NO</td>
<td>Matrix metalloprotease-2</td>
</tr>
<tr>
<td>Osteopontin</td>
<td>Cytokine-stimulated NO</td>
</tr>
</tbody>
</table>

NO, nitric oxide.
as angiotensin II) (5, 28). In fact, ET-1 stimulates MC proliferation in an autocrine fashion because antisense oligonucleotides to ET-1 reduce spontaneous MC proliferation in vitro (67). The mitogenic effects of ET-1 in MCs are likely primarily mediated via ETRA (28, 105), although there are data suggesting that activation of ETRB, as well as ETRA, in human MCs can induce proliferation (32, 80) (Fig. 3).

ET-1 stimulation of MC proliferation involves several pathways, among which MAPK figures prominently (83, 106). ET-1 induces activity of all three major MAPK cascades in MCs: ERK1 and ERK2 (112), JNK/SAPK (2), and p38 MAPK (99). In addition, ET-1 stimulates expression of MAPK phosphatase 1 (MKP-1) (23), a dual-specificity phosphatase that downregulates MAPK signaling. There is convincing evidence that ET-1 induces cell proliferation primarily via activation of the Ras-Raf-Mek-ERK-signaling cascade (92) (Fig. 2). Late-onset, stable changes in gene expression, associated with MC hypertrophy, were recently reported to be controlled by ET-1-mediated activation of ERK, JNK/SAPK, and phosphatidylinositol 3-kinase pathways (30). In addition, ET-1 activation of ERK1/2 is partially dependent on tyrosine phosphorylation of the epidermal growth factor receptor and likely involves caveolin-1 (37). ET-1 induction of MAPK and subsequent increased cyclin-dependent kinase 4 and cyclin D1 expression occur through activation of ETRA (45, 105).

ET-1 rapidly enhances tyrosine phosphorylation of proline-rich tyrosine kinase 2 (Pyk2) and the Src family of cytoplasmic tyrosine kinases. ET-1-induced activation of Pyk2 and Src results in tyrosine phosphorylation of multiple signaling molecules, including recruitment of the adaptor proteins Shc and Grb2 that ultimately lead to cell proliferation and/or hypertrophy (Fig. 2). The ubiquitously expressed adaptor protein Shc (84), which exists in three isoforms with relative molecular masses of 46, 52, and 66 kDa (p46Shc, p52Shc, and p66Shc, respectively), plays an important role in ET-1 signaling. ET-1 treatment of MCs results in persistent tyrosine phosphorylation of p52Shc (23), which promotes the association of p52Shc with the Grb2/Sos complex due to recognition of p52Shc P-Tyr by the Grb2 SH2 domain. The formation of the trimeric module Shc/Grb2/Sos localizes the guanosine exchange factor Sos to GTPase Ras, causing the switch of RasGDP into the active GTP-bound form. This initial activation of Ras is followed by rapid inactivation of Ras, as a direct consequence of the MEK/ERK-dependent Sos1 phosphorylation and Sos1 release from the trimeric module. Subsequent to Ras and ERK deactivation, Sos1 reverts to the nonphosphorylated condition, enabling it to bind again to the Grb2/Shc complex, which is stabilized by persistent Shc phosphorylation, resulting in biphasic activation of Ras (23). The second peak of ERK activation is presumably attenuated by activation of a dual-specificity phosphatase.

ET-1 also induces serine phosphorylation of p66Shc via activation of the MEK/ERK-signaling module, resulting in p66Shc association with the serine-binding, motif-containing protein 14—3—3 (24). Interestingly, p66Shc+/− mice are resistant to oxidative stress, and the p66Shc-mediated response to oxidative stress is dependent on serine phosphorylation (71). So far, ET-1 is among few physiological agonists shown to induce serine phosphorylation of p66Shc, raising the possibility that ET-1 is involved in cellular resistance to oxidative stress.

Cell Contraction

ET-1 potently stimulates MC contraction, an effect that is independent of dihydropyridine-sensitive Ca2⁺ channels and is likely mediated through ETRA activation (3, 91, 102). Pyk2 may play a crucial role in ET-1-mediated contraction of MCs because 1) it is the only cytoplasmic tyrosine kinase activated by mobilization of intracellular Ca2⁺ (66); 2) tyrosine phosphorylation appears to be essential for the contractile effects of many G protein-coupled receptor ligands (70, 108, 113, 122); 3) ET-1 stimulates Pyk2 autophosphorylation in a Ca2⁺-dependent manner in MCs (99); and 4) Pyk2 is responsible for p38 MAPK activation in MCs, which has been implicated in MC contraction (99). It should be noted that ET-1-mediated contraction also involves activation of the Rho/Rho kinase pathway because Rho/Rho kinase inhibition markedly blunts
ETRB agonist-induced vasoconstriction (9). ET-1-induced MC contraction may also involve Src tyrosine kinases, possibly via β-arrestin-1-mediated recruitment of Src to a molecular complex with the endothelin receptor (43) and/or adhesion-dependent activation of Src via interaction with focal adhesion kinase (FAK) (10). ET-1 may activate Src, at least in part, via activation of Ca$^{2+}$/calmodulin-dependent protein kinase II (CaMK II) in MCs (111) (Fig. 2).

ET-1 stimulation of MC contraction may be modified by vasorelaxant substances, particularly nitric oxide (NO) and PGE$_2$. Activation of ETRB increases cGMP via induction of NO, an effect that is dependent on release of Ca$^{2+}$ from intracellular stores and calmodulin (81) (Fig. 3). In contrast, ET-1 inhibits induction of cytokine-stimulated inducible NOS (iNOS) activity via activation of ETRA (7, 33). ET-1 also stimulates cyclooxygenase (COX) activity, resulting primarily in increases in PGE$_2$ with small increases in thromboxane A$_2$ (95). This occurs via ETRA induction of PLA$_2$ (26) and COX-2 (38). COX-2 stimulation depends on intracellular Ca$^{2+}$ release, Ca-calmodulin kinases, and non-receptor-linked protein tyrosine kinase activity (15) and is independent of PKC (26). Recent studies also demonstrate that ET-1 enhances nuclear factor of activated T cell translocation to the nucleus in MCs and increases nuclear factor 2 of activated T cell binding to the COX-2 promoter (101).

**Extracellular Matrix Accumulation**

ET-1 increases fibronectin (28, 82), type IV collagen (28, 114), and type I collagen (82) synthesis by MCs (Table 2). In addition, ET-1 partially mediates angiotensin II-mediated MC extracellular matrix accumulation (28). These effects are primarily mediated via ETRA (28, 55) (Fig. 3) with resultant activation of PKC-β, -δ, -ε, and -ζ (28, 114). Induction of fibronectin and type I collagen synthesis appears to involve ERK2, but not JNK or p38 MAPK (82).

ET-1 also seems to have a net inhibitory effect on extracellular matrix degradation by MCs. Although ET-1 has been described to increase MC collagenase (92) and matrix metalloproteinase-2 activity (MMP-2) (55), the peptide has been shown to reduce fibrinolytic activity (via stimulation of plasminogen activator inhibitor production) (47), to reduce basal and cytokine-stimulated MMP-2, and to enhance secretion of tissue inhibitor of MMP-2 (117). These latter effects are also mediated by activation of ETRA (117) (Fig. 3). Notably, mice transgenic for the ET-1 promoter and gene developed marked glomerulosclerosis in the absence of elevations in systemic blood pressure (90).

**PATHOPHYSIOLOGY OF ET-1 IN MCS**

**Glomerulonephritis**

To demonstrate that MC-derived ET-1 is of pathogenic importance in glomerulonephritis (GN), it is necessary to show that its production is increased, that this results in a pathophysiological effect, and that blockade of ET-1 action ameliorates disease severity. As discussed above, inflammatory cytokines increase MC ET-1 release in vitro, suggesting that MC ET-1 production should be enhanced in GN. This has indeed been shown in animal models: MC ET-1 production is increased in rat models of immune complex GN (87), nephrotoxic serum nephritis (120), and mesangial proliferative GN (119). In addition, MC ET-1 production is augmented in human systemic lupus erythematosus (32), urinary ET-1 excretion is proportional to the severity of human mesangial proliferative GN (18), and N-formyl-Met-Leu-Phe-stimulated neutrophils from patients with IgA nephropathy stimulate rat MC ET-1 release more than neutrophils from patients with non-IgA mesangial proliferative GN (11).

As discussed earlier, there is abundant evidence that ET-1 increases MC proliferation and matrix accumulation. ET-1 may further stimulate inflammation because it elevates human MC production of TNF, ICAM-1, and VCAM-1 (14). Furthermore, ET-1 reduces TNF-mediated apoptosis in MCs (via induction of COX-2), potentially causing additional cell accumulation (46). That these effects of ET-1 are of pathophysiological relevance is borne out by studies using ET antagonists (Table 3). Indirect evidence comes from studies in which either an angiotensin-converting enzyme inhibitor (ACEI) (87) or a thromboxane A$_2$ receptor blocker (120) reduced glomerular injury in rat models of GN associated with decreased MC ET-1 levels. More direct evidence is afforded by several studies. The ECE inhibitor CGS-26303 reduces MC expansion in purumycin aminonucleoside (PAN) nephrosis in rats (22), and an ETRA antagonist, FR-139317, reduces glomerular collagen α3(IV) and laminin accumulation in PAN nephrosis (20). In rat models of mesangial proliferative GN, FR-139317 reduced MC proliferation, combined ETRA/ETRB blockade with bosentan improved renal function and reduced MC ET-1 mRNA levels (27), and antisense oligonucleotides to ET-1 decreased MC proliferation and matrix expansion (67). Thus there is strong evidence that MC-derived ET-1 plays a significant pathogenic role in GN.

**Diabetes Mellitus**

Although there is no direct evidence in vivo that MC ET-1 production is increased in diabetic nephropathy, there are in vitro data suggesting that MC ET-1 synthesis is increased by hyperglycemia. Furthermore, hyperglycemia modifies MC responses to ET-1. For example, ET-1-stimulated p38 MAPK and cAMP-responsive element-binding protein phosphorylation in MCs is enhanced by hyperglycemia (109). In addition, hyperglycemia augments ET-1-stimulated α$_1$(IV) collagen production in MCs, an effect that requires PKC-δ, -ε, -ζ, and ERK1/2, as well as PKC-α and -β (latter independent of ERK1/2) (36). Interestingly, hyperglycemia decreases the MC ET-1 Ca$^{2+}$ signal through decreased receptor-operated Ca$^{2+}$ influx (79), an effect that could explain hyperglycemic inhibition of ET-1-induced MC contraction (16).
There is abundant evidence in vivo that ET-1 antagonists ameliorate glomerular injury in animal models of diabetic nephropathy (Table 3). ET-1 blockade with combined ETRA/ETRB inhibition (bosentan) reduced albuminuria and increased glomerular filtration rate (GFR) (12) and ameliorated mesangial matrix, fibronectin, and α5(IV) collagen accumulation in diabetic rats (13). In addition, combined receptor blockade with LU-224332 for 36 wk prevented fibronectin and collagen IV accumulation in diabetic rats (35). The beneficial effects of combined blockers may be largely a result of ETRA blockade because 1) ETRA blockade with YM-598 reduced albuminuria in a diabetic rats (100); 2) ETRA blockade with LU-135252 for 6 mo reduced glomerular histological injury in rats with streptozotocin-induced diabetes (17); and 3) ETRA blockade with FR-139317 for 24 wk decreased glomerular mRNA levels of collagen, laminin, TGF-β, basic FGF, and PDGF-B in diabetic rats (75). Taken together, these studies suggest that MC ET-1 production is enhanced in diabetic nephropathy and that excessive ET-1 action in the diabetic glomerulus may cause enhanced matrix accumulation, proteinuria, and reduced GFR.

**Hypertensive Glomerulosclerosis**

MC ET-1 production may be elevated in hypertension. MC ET-1 levels are higher in spontaneously hypertensive (SHR) rats than in nonhypertensive controls (64), whereas MC ETRA mRNA levels are higher in stroke-prone SHR compared with normotensive Wistar-Kyoto (WKY) rats (34). Several agents also enhance MC ET-1 release more from SHR than WKY animals, including angiotensin II, phenylbiguanide ester, vaso-pressin, thrombin, and PDGF (41, 42). Relevant to these latter findings is the observation that ACEI reduced ET-1 production by MCs from uninephrectomized SHR rats (64). Although data are limited on the role of MC ET-1 in hypertensive glomerular injury, one study found that bosentan reduced glomerular extracellular matrix accumulation in Nω-nitro-L-arginine methyl ester-induced hypertensive mice despite the lack of an effect on blood pressure (8). This study also found that bosentan normalized the activity of an α5(1) collagen promoter-luciferase transgene in these mice. Thus initial studies suggest that MC-derived ET-1 may also play a role in hypertensive glomerulosclerosis.

**Acute and Chronic Renal Failure**

Data on MC ET-1 production or actions in chronic renal failure outside of the specific examples above are few, although there is abundant evidence that ET-1 plays a role in the progression of renal insufficiency (54, 57). There is also little information on MC-derived ET-1 in acute renal failure. Numerous studies have shown that ET-1-induced vasoconstriction is of pathogenic importance in various forms of acute renal failure (78); however, it is unclear whether this ET-1 substantially derives from MCs. Agents that induce renal vasoconstriction and/or cause acute renal failure can stimulate MC ET-1 release, including cyclosporin, FK-506, myoglobin, thromboxane A2, angiotensin II, vaso-pressin, and reactive oxygen species (29, 39, 53, 59, 62, 88, 121). It is conceivable that MC-derived ET-1 in the setting of acute renal failure reduces the glomerular ultrafiltration coefficient by eliciting MC contraction or even contributes to downstream efferent arteriolar vasoconstriction; however, these considerations are entirely conjectural.

**ET ANTAGONISTS IN THE CLINICAL SETTING**

Given the strong indictment of MC ET-1 overactivity in the pathogenesis of the three leading causes of end-stage renal disease, namely, diabetes, hypertension, and glomerulonephritis, there is cause for considerable excitement about the used of ET-1 blockers in the treatment of these diseases. With the recent Food and Drug Administration approval of an ET antagonist...
for treatment of primary pulmonary hypertension, the stage is now set for realistic consideration of the use of this class of agents in treating kidney disease. To date, there have been no clinical studies that have addressed this issue; however, studies of other diseases provide an impetus for the translation of ET blockers to the therapy for renal disease. This section will review the present status of ET antagonists in the clinical setting, focusing on the cardiopulmonary system. While this material does not cover diseases of the kidney, we thought it important at this time to emphasize the work being done in other organ systems, hopefully as an impetus for similar studies to be conducted in renal disease.

Initial studies focused on the effects of oral combined ETRA/ETRB blockade with generally disappointing results. One of the earliest studies was a phase II trial examining the effect of 4 wk of therapy with the combined ETRA/ETRB-receptor antagonist bosentan on blood pressure in 293 patients with mild to moderate essential hypertension (63). Blood pressure decreased modestly, and there were a significant number of side effects, including headache, flushing, leg edema, and reversible elevations in liver enzymes (LFTs). Bosentan was then used in a trial of 370 patients with New York Heart Association (NYHA) class IIIb/IV congestive heart failure (CHF), the so-called Research on ET Antagonism in CHF (40). The trial was discontinued prematurely because of elevations in LFTs; however, it appeared that bosentan initially worsened CHF but may have slightly improved the outcome after 6 mo. Subsequently, a lower dose of bosentan was administered to 1,613 patients with NYHA class IIIb/IV CHF in the ENt Antagonist Bosentan for Lowering Cardiac Events in Heart Failure trial (ENABLEI in Europe and ENABLE2 in North America) (51). Unfortunately, no difference was detected between bosentan and placebo on all-cause mortality or hospitalization for CHF. Another trial, the ENrastentan Clinical Outcomes Randomized study, using a combined ETRA/ETRB antagonist, found that ET blockade increased heart failure events in 419 patients with NYHA class II/III CHF (85). An intravenous ETRA/ETRB blocker, tezosentan, has been given in a trial involving 285 patients with acute decompenated CHF (RITZ-2), and there was decreased pulmonary capillary wedge pressure and increased cardiac output (68). Unfortunately, a follow-up study (RITZ-3) found no affect of tezosentan on the outcome of acute pulmonary edema (52).

Another phase II study, using TAK-044, a combined ETRA/ETRB blocker, found no difference in clinical outcomes in patients with aneurysmal subarachnoid hemorrhage (89). The most encouraging results using combined ETRA/ETRB blockers have been in pulmonary hypertension. Bosentan modestly increased exercise capacity in 213 patients; however, 9 patients stopped the drug because of side effects (86). Finally, there is a case report of a patient with systemic sclerosis whose digital ulcers and cutaneous fibrosis substantially improved with 1 yr of bosentan therapy (40). In summary, the clinical trials with ETRA/ETRB combined blockade in cardiopulmonary disease have either been unpromising or shown only a modest benefit.

ETRA-specific antagonists could be potentially superior to combined blockers by virtue of avoiding inhibition of NO, or other factor, production as a result of ETRB blockade. To date, relatively few clinical studies have been performed using these agents. Treatment with darusentan (LU-135252) for 6 wk reduced mean blood pressure (up to 11 mmHg) in a trial of 392 patients with moderate essential hypertension (76). A 3-wk trial (HEAT) with oral darusentan in 157 patients with NYHA class III CHF showed improved cardiac index and reduced systemic vascular resistance, with no change in LFTs (69). Treatment with sitaxsentan for 12 wk in 20 patients with NYHA class II-IV pulmonary hypertension improved exercise capacity (6). Interestingly, a phase II study using ETRA blockade in patients with prostate cancer (prostate cancer cell lines express very high levels of ETRA) is underway (85).

Finally, agents are being developed that inhibit ET actions as well as affect other systems. BMS-248360, a potent inhibitor of ETB and AT1 receptors, has recently been described (73). ECE inhibitors have been designed, although they have not been clinically tested. Because ECEs share 37% sequence homology with neutral endopeptidase (NEP), which degrades atrial natriuretic peptide and bradykinin, dual ECE and NEP inhibitors have been designed. One such dual inhibitor, CGS-26303, reduces glomerular lesions in rats with PAN nephrosis (22). Even triple inhibitors (ECE, NEP, and ACE) have been designed: all three enzymes are zinc metalloproteases and can be inhibited by groups that interact with the zinc-binding domain (e.g., sulphydryl groups). One such triple inhibitor, SCH-54470, decreased blood pressure and proteinuria and increased GFR in the remnant kidney rat model (110). Last, vasopeptidase inhibitors (inhibiting ACE and NEP) have been designed (e.g., omapatrilat); however, their clinical utility is uncertain (107). Notably, inhibition of NEP can result in elevated ET-1 levels because this metalloprotease degrades ET-1.

In summary, the stage is set for clinical trials of ET inhibitors in patients with glomerular disease characterized by increased ET-1 production and actions. The challenges include finding an agent with tolerable therapeutic indexes, targeting ET receptors that most likely are pathogenic (likely ETRA), and, most of all, meeting the challenges of conducting studies whose benefit may only be truly known after long-term drug administration.

CONCLUSION

In conclusion, abundant evidence points to a pivotal role for ET-1 in the biology, and particularly the pathology, of the renal mesangium. The peptide is produced by MCs and can, in turn, act on MCs to elicit proliferation, hypertrophy, contraction, and/or extracellular matrix accumulation. These effects are mediated in large part through activation of ETRA and...
particularly involve PKC and MAPK. Excessive ET-1 production by, and action on, MCs is of pathogenic importance in glomerular damage in animal models of GN, diabetes, and hypertension. With the emergence of Food and Drug Administration approval of clinical ET antagonists, the time is propitious for clinical trials using ET antagonists in these renal diseases. While challenges exist with such trials, it is our contention that the preclinical studies are so strongly indicative of a potential beneficial effect of these agents in glomerular disease that this challenge should be aggressively pursued.

DISCUSSIONS

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REFERENCES


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