Endothelin Receptors and Their Antagonists

Janet J. Maguire, BSc, PhD, and Anthony P. Davenport, BSc, PhD, MA, DIC

Summary: All three members of the endothelin (ET) family of peptides, ET-1, ET-2, and ET-3, are expressed in the human kidney, with ET-1 being the predominant isoform. ET-1 and ET-2 bind to two G-protein–coupled receptors, ETA and ETB, whereas at physiological concentrations ET-3 has little affinity for the ETA receptor. The human kidney is unusual among the peripheral organs in expressing a high density of ETB. The renal vascular endothelium only expresses the ETB subtype and ET-1 acts in an autocrine or paracrine manner to release vasodilators. Endothelial ETB in kidney, as well as liver and lungs, also has a critical role in scavenging ET-1 from the plasma. The third major function is ET-1 activation of ETB in the nephron to reduce salt and water reabsorption. In contrast, ETA predominate on smooth muscle, causing vasoconstriction and mediating many of the pathophysiological actions of ET-1. The role of the two receptors has been delineated using highly selective ETA (BQ123, TAK-044) and ETB (BQ788) peptide antagonists. Nonpeptide antagonists, bosentan, macitentan, and ambrisentan, that are either mixed ETA/ETB antagonists or display ETA selectivity, have been approved for clinical use but to date are limited to pulmonary hypertension. Ambrisentan is in clinical trials in patients with type 2 diabetic nephropathy. This review summarizes ET-receptor antagonism in the human kidney, and considers the relative merits of selective versus nonselective antagonism in renal disease.

Keywords: Ambrisentan, antagonist, bosentan, endothelin-1, macitentan, sitaxentan

All three members of the endothelin (ET) family of peptides, ET-1, ET-2, and ET-3, are expressed in the human kidney, although ET-1 is the predominant isoform. ET-1 and ET-2 bind to two G-protein–coupled receptors, ETA and ETB, whereas at physiological concentrations ET-3 has little affinity for the ETA receptor. The endothelin receptors are members of the Family A G-protein–coupled receptors, a class of proteins that has been exploited very successfully as targets for the development of drugs.

The human kidney is unusual among the peripheral organs in expressing a high density of ETB. The renal vascular endothelium only expresses the ETB subtype and ET-1 acts in an autocrine or paracrine manner to release vasodilators. Endothelial ETB in kidney, as well as liver and lungs, has a critical role in scavenging ET-1 from the plasma. The third major function is for ET-1 activation of ETB in medullary epithelial cells to reduce salt and water reabsorption. ETA predominate on the vasculature to cause vasoconstriction. The pathophysiological actions of ET-1 are mediated mainly via the ETA subtype. The role of the two subtypes has been delineated in preclinical and acute experimental studies using highly selective ETA (including BQ123, TAK-044) and ETB (BQ788) peptide antagonists. Three nonpeptide antagonists, bosentan, macitentan, and ambrisentan, that are either mixed ETA/ETB antagonists or display ETA selectivity, have been approved for clinical use, primarily in pulmonary arterial hypertension.

In renal pathophysiological conditions ET-1 contributes to vascular remodeling, proliferation of mesangial cells, and extracellular matrix production, mainly through binding to ETA. Beneficial actions of ET-1 on sodium and water regulation mainly are ETB-mediated. These findings suggest an ETA-selective antagonist would have a therapeutic advantage over a mixed antagonist in renal disease. Acute studies directly comparing mixed and selective peptide antagonists suggest selective ETA blockade, however, sparing ETB may be beneficial. However, this was balanced by a greater prevalence of side effects for small-molecule, orally active ETA antagonists compared with mixed antagonists, although the latter also have their limitations. The ET signaling pathway in the kidney remains a promising clinical target for receptor antagonism, which may be realized by the next generation of antagonists.

ET RECEPTORS

The ET family comprises three isoforms, ET-1, ET-2, and ET-3.1,2 Although messenger RNA encoding all three has been detected in human kidney, ET-1 is the predominant intrarenal isoform.3 ETs interact with two
distinct G-protein–coupled receptors, ET<sub>A</sub> and ET<sub>B</sub> (Fig. 1), which were identified 2 years after the discovery of the endogenous peptides in 1988. They are both class A, G-protein–coupled receptors; this class is the target of nearly half of currently available medicines. This has resulted from well-developed medicinal chemistry strategies and high-throughput screening programs to identify small-molecule drugs, stimulating considerable effort to discover ET-receptor antagonists. The initial clue to the existence of two subtypes and the key to classifying the receptors was that ET-1 and ET-2 are equipotent at the ET<sub>A</sub> subtype whereas ET-3 shows at least 100-fold lower potency and at physiological concentrations ET-3 is unlikely to activate this subtype (Table 1). All three ETs bind to ET<sub>B</sub> with similar affinity. This review focuses on the role of ET receptors in the human kidney and considers the clinical pharmacology of ET antagonists that have been used to block these receptors. The effects of ET-1 on the kidney are complex and more detailed information can be found in reviews on renal endothelin physiology and pathology, and the pharmacology of the endothelin signaling pathway.

**NO EVIDENCE FOR FURTHER ET-RECEPTOR SUBTYPES**

Further receptor subclassifications have been proposed including suggestions that ET<sub>B</sub> could be subdivided into ET<sub>B1</sub>, present on endothelial cells, and ET<sub>B2</sub> on smooth muscle cells, but there currently is no evidence that the receptors expressed by these two cell types can be distinguished pharmacologically. ET-receptor antagonists have not been successful in certain conditions such as heart failure, perhaps implying that ETs may mediate...
their actions via previously unsuspected receptors; however, this is unlikely. Following the sequencing of the human genome, it is accepted that all genes that potentially encode a G-protein-coupled receptor have been identified and currently are classified as ‘orphan’ to indicate that their endogenous ligand is not yet known.13,14 These remaining orphan receptors (approximately 80) have been screened against more than 20 ET peptides (including all three endogenous isoforms and their corresponding big ET precursors, C-terminal metabolites, the ETA antagonist BQ123, and the ETB agonist BQ3020) without detectable binding. The screen also included two of the most closely related orphan receptors to ETA and ETB, GPR37 (also known as endothelin-receptor type B-like receptor or Parkin-associated endothelin receptor-like receptor) and its related receptor GPR37L1. Two neuropeptides, prosaptide and prosaposin, that are structurally distinct from the ETs have been suggested to be the endogenous ligands for GPR37 and GPR37L1.15

PEPTIDE AGONISTS

Experimental medicine studies in volunteers mainly use ET-1 that is equipotent for ETA and ETB (Table 1). ET-3, which is modestly selective for ETB,7 also has been used but greater ETB selectivity is shown by sarafotoxin S6c, one of the isoforms originally identified from snake venom.16 IRL1620 (Suc-[Glu9,Ala11,15]-endothelin-18-21)17 is a truncated linear analogue in which the N-terminus has an N-succinyl modification, reducing metabolism by non-specific peptidases. It was developed as an ETB agonist but now is used in clinical trials as a potential vasodilator in the delivery of anticancer agents and in neuroprotection where it is known as SPI-1620 (licensed by Spectrum Pharmaceuticals, Henderson, NV). The second widely used ETB agonist is BQ3020 ([Ala11,15]Ac-ET-l6-21),18 however, this compound has not been used clinically.

PEPTIDE ANTAGONISTS

The first endothelin-receptor antagonists to be discovered were from natural product screening, compound libraries, or drug design based on the structure of the endogenous ET peptides (Table 1). The most widely used, according to the number of published articles, is the cyclic pentapeptide BQ123 (D-Asp-L-Pro-D-Val-L-Leu-D-Trp-) (Ihara et al19), based on peptides isolated from Streptomyces misakiensis, a highly selective competitive ETA antagonist with low nanomolar affinity for the receptor. The second most widely used is FR 139317 (N-[(hexahydro-1-azepinyl) carbonyl]L-Leu[1-Me]D-Trp-3 [2-pyridyl]-D-Ala),20 a
linear tripeptide. These are both highly ET<sub>A</sub> selective for human (as well as rodent) ET receptors and at concentrations used in experimental medicine or in vivo animal experiments are likely to block only the ET<sub>A</sub> receptor; data from these studies can be interpreted with confidence. TAK-044 is a cyclic hexapeptide also isolated from <i>S. misakienis</i> with a more modest degree of ET<sub>A</sub> selectivity.<sup>21</sup> BQ788 (N-[2R,6S]-2,6-dimethyl-1-piperidinyl)carbonyl]-4-methyl-L-leucyl-N-[1R]-1-carboxylatopentyl]-1-([methoxycarbonyl]-D-tryptophanamide) is a modified tripeptide developed by structure-activity analysis<sup>22</sup> and is a selective competitive ET<sub>B</sub> antagonist (usually showing one to two orders of magnitude selectivity for ET<sub>B</sub> over ET<sub>A</sub>) in human beings and across species. Because these compounds are all peptides, they have little or no oral bioavailability, require intra-arterial administration, and are metabolized or excreted over comparatively short periods of time. An advantage in their use is that they are soluble and do not bind plasma proteins. Therefore, they are used for short-term, acute investigations in both animal models and in experimental medicine studies.

**ET<sub>A</sub> RECEPTORS PREDOMINATE ON SMOOTH MUSCLE OF RENAL VESSELS AND MEDIATE VASCOCONSTRICTION**

A major physiological action of ET-1 is to function as one of the most powerful vasoconstrictors of human blood vessels. As such, ET-1 plays a major role in regulating vascular function in all organ systems, including the kidney (Fig. 1). As in other vessels, ET-1 is thought to be released from endothelial cells lining intrarenal vessels throughout the cortex and medulla. In the human vasculature, including that of the kidney, under normal physiological conditions release of ET-1 from endothelial cells causes sustained vasoconstriction via ET<sub>A</sub> that predominate on the underlying smooth muscle. Under pathophysiological conditions in which ET-1 is overproduced, vascular cells also may undergo proliferation and contribute to vascular remodeling and the development of renal fibrosis. Figure 1 shows the ratio of the densities of the two receptor subtypes measured by radioligand binding assays with the ET<sub>A</sub> subtype representing greater than 90% of ET receptors in the smooth muscle layer of all renal vessels studied. This includes the large conduit vessels, the arcuate arteries, and veins at the corticomedullary junction, as well as small intrarenal vessels such as the afferent and efferent vessels of the glomerulus.<sup>23–27</sup>

In a detailed study using human isolated main stem renal arteries and veins in organ baths,<sup>28</sup> ET-1 was, as expected, a potent vasoconstrictor, with the concentration producing half-maximal response (EC<sub>50</sub>) values of 4 and 1 nmol/L, respectively. In renal artery, ET-3 and the ET<sub>B</sub> agonist sarafotoxin 6c showed little or no activity up to 300 nmol/L. In veins, some but not all samples responded to ET-3, but this peptide was much less potent than ET-1, consistent with an ET<sub>A</sub>-mediated action. Interestingly, S6c concentration-related contractions were found in some individuals and, although more potent than ET-1, the maximum response was 30% to 60% of that obtained with ET-1. Crucially, however, the ET<sub>A</sub> antagonist BQ123 fully reversed the ET-1 contractions in both arteries and veins without reducing the maximum agonist response, consistent with a competitive antagonist. Therefore, in renal vessels the endogenous peptides ET-1 and ET-3 appear to mediate vasoconstriction via the ET<sub>A</sub>, indicating that ET<sub>B</sub>-mediated responses in human renal vessels are of little importance. The pharmacology of isolated renal arteries and veins is similar to vessels obtained from other human vascular beds, with ET<sub>A</sub> antagonists fully reversing an ET-1 response.<sup>29</sup> This is critical to understanding the importance of selectivity for the two subtypes. Sarafotoxin S6c-induced constrictor responses have been used previously as evidence of significant ET<sub>B</sub> constrictor responses in human vessels. However, it is not an endogenous ligand and ET-1 responses are fully reversed using ET antagonists. Bohm et al<sup>30</sup> performed key experimental medicine studies that showed in volunteers in vivo that BQ123 inhibited the ET-1-mediated increase in renal vascular resistance whereas BQ788 (ET<sub>B</sub> antagonist) potentiated the ET-1 effect, implying a constrictor role for ET<sub>A</sub> and that ET<sub>B</sub> clears ET-1 from the plasma. Kaasjager et al<sup>31</sup> also concluded that the systemic and renal vasoconstrictor effects of ET-1 in human beings are mediated by the ET<sub>A</sub>.

A further unusual feature of ET-1 compared with other vasoconstrictors is that the constrictor response is sustained over a considerable period of time, lasting for several hours or in some cases several days.<sup>32</sup> Contractions compared with many other vasoconstrictors are slow to wash out, which is consistent with a slow dissociation rate for ET-1 and may contribute to sustained hypertension and/or ET-induced vasospasm associated with pathophysiological conditions such as chronic kidney disease. Importantly, ET antagonists are able to relax ET<sub>A</sub>-mediated vasoconstriction in vessels preconstricted with ET-1<sup>33</sup> and this may reflect rapid internalization of the ligand receptor complex for recycling to the membrane (Fig. 1). In contrast, binding of ET-1 to ET<sub>B</sub> in vivo often is not displaced by ET<sub>B</sub> antagonists,<sup>34</sup> which is in agreement with ET<sub>B</sub> being internalized by a different pathway and degraded in the lysosome.

**HOW IMPORTANT IS THE SMALL POPULATION OF ET<sub>B</sub> RECEPTORS EXPRESSED BY VASCULAR SMOOTH MUSCLE?**

In some, but not all, human vessels, a small population of ET<sub>B</sub> (usually <15%) can be measured by ligand binding. Although in some human beings isolated renal vessel responses to high concentrations of ET-3 were...
detected, comparison of equipotent concentrations of ET-3 and ET-1 in healthy volunteers found that ET-3 had no effect on blood pressure or renal hemodynamics, which might have been expected if ET_B contributed significantly to a contractile response. Whether the proportion of vascular ET_B changes with disease remains controversial and has not been studied in detail in pathophysiological renal tissue. However, detailed studies in vitro in human coronary arteries with atherosclerotic lesions did not show any increase. In agreement, in experimental medicine studies in both heart failure patients and volunteer controls, selective ETA antagonism (BQ123) caused the expected potent vasodilatation in the peripheral circulation. However, BQ788 caused vasoconstriction in both groups, consistent with blocking endothelial cell ET_B-mediated vasodilatation, with no evidence of contractile ET_B.

OTHER CELL TYPES EXPRESSING ETA RECEPTORS

ETA have been shown to be present on human and rat podocytes (glomerular epithelial cells) that wrap around the capillaries of the glomerulus within Bowman’s capsule. Ortmann et al also detected messenger RNA encoding ET_B as well as ETA on human podocytes. However, ETA contribute to podocyte injury through cytoskeleton disruption and apoptosis and only ETA antagonists are effective in preventing podocyte injury. In renal disease, proliferation in mesangial cells, extracellular matrix production, and inflammation are mediated mainly by ETA.

ETB RECEPTORS PREDOMINATE IN THE KIDNEY AND MEDIATE BENEFICIAL VASODILATATION, CLEARING OF ET-1 FROM PLASMA, AND NATRIURESIS

In peripheral tissues such as the heart (Fig. 1), ETA are more abundant (> 60%) than ETB (Fig. 2). In marked contrast, in the kidney, lungs, and liver this ratio is reversed. Although measurements of receptors within smooth muscle throughout the renal vasculature show a predominance of ETA, 70% of the ET receptors in both cortex and medulla in human kidney are ETB. ETB predominate, reflecting, at least in part, that these are endothelial cell–rich tissues similar to liver and lungs. Endothelial cells line every vessel wall and have a mass comparable with other endocrine organs. Although ETB also are expressed by other cell types, selective deletion of the endothelial cell ETB, leaving ETB on other cells intact, shows that in many organs, including the kidney, liver, and lungs, endothelial cells represent the majority of the receptors.

A consensus has emerged that ETB mediates vasodilatation by the release of endothelium-derived relaxing factors (nitric oxide, prostacyclin, and/or endothelium-derived hyperpolarizing factor), acting as a feedback mechanism to limit the vasoconstrictor action of ET-1. Infusions of ET-1 into the brachial artery of volunteers produces a biphasic response: low doses of ET-1 cause ET_B-mediated vasodilatation, however, as the concentration increases to higher pathophysiological concentrations, vasodilatation is overwhelmed by ETA-mediated constrictor responses. When endothelial dysregulation occurs in renal disease there is a loss of opposing vasodilators, leading to increased vasoconstriction and vasospasm.

Endothelial cell ETB function as scavenging or clearing receptors to remove ET-1 from the circulation, particularly by the ETB-rich tissues: kidney, lungs, and liver. Selectively blocking ETA, but not ETB, with a low dose of the peptide antagonist TAK-044 infused into volunteers caused no change in measured plasma ET-1 levels. However, a higher dose that blocked both subtypes increased ET-1...
levels by more than three-fold as a result of reducing clearing by ETβ.\textsuperscript{45}  
In renal circulation, in agreement with other vascular beds in human beings, systemic infusion of ET-1, which activates both receptors, into volunteers increased blood pressure (6 mm Hg), and decreased renal plasma flow, glomerular filtration rate, and sodium excretion rate.\textsuperscript{31} Although BQ123 infused alone did not affect basal arterial blood pressure or renal or splanchic vascular resistance, the antagonist inhibited the increase in vascular resistance induced by co-infusion of ET-1. In contrast, BQ788 alone caused the opposite effect: increased renal or splanchic vascular resistance, consistent with blocking endothelial cell–receptor vasodilatation. Second, BQ788 potentiated the ET-1–induced increase in vascular resistance mediated by ET\textsubscript{A}, suggesting that blocking the scavenging receptors modulated plasma ET-1 levels.\textsuperscript{30} Inhibition of tonic nitric oxide production by inhibition of nitric oxide synthase elicits vasoconstriction with an increase in mean arterial pressure and vascular resistance in many organs, including the kidney. Renal and systemic vasoconstriction in volunteers caused by the nitric oxide synthase inhibitor N-nitro-L-arginine methyl ester were attenuated by BQ123, supporting the concept that the balance between endogenous nitric oxide production and ET-1/ET\textsubscript{A} activity contributes to renal and systemic tone in human beings.\textsuperscript{46,47}  
The role of ET\textsubscript{B} clearing receptors has been studied in detail in endothelial cell–specific ET\textsubscript{B} knock-out mice. In these animals, clearance of an intravenous bolus of labeled ET-1 was reduced significantly compared with wild-type controls. Importantly, functioning ET\textsubscript{B} were retained on all other cell types such as epithelial cells.\textsuperscript{43,48} Dynamic imaging of rats using positron emission tomography showed that after infusion of \textsuperscript{18}F ET-1, there was remarkably fast clearance of the radioligand from the circulation (plasma-half life (t\textsubscript{1/2}) = 0.43 min), with high levels of radioligand accumulated in the kidney, liver, and lung, which rapidly reached equilibrium, and this was maintained for at least 20 minutes. Infusion of BQ788 before injecting \textsuperscript{15}F ET-1 reduced the amount of radioligand visualized in the lung and kidney by 85% and 55%, respectively, consistent with blockade of ET\textsubscript{B}. However, infusion of BQ788 after \textsuperscript{15}F ET-1 did not displace the bound ligand.\textsuperscript{34} This finding is consistent with the internalization of the ligand-receptor complex to the lysosome where ET-1 is thought to be degraded, similar to other peptides, by cathepsin A. In support, cathepsin A knock-out mice showed reduced ET-1 degradation and significantly increased arterial blood pressure.\textsuperscript{49} Inactivation of ET-1 by kidney, liver, and lungs may be particularly important for ET-1 because it is structurally unusual compared with other vasoactive peptides, possessing two disulfide bridges that confer resistance to degradation by nonspecific peptidases.  
ET-1 promotes diuresis and natriuresis via ET\textsubscript{B} located on epithelial cells throughout the tubular epithelium, particularly the inner medullary collecting duct cells.\textsuperscript{50} Deletion of ET\textsubscript{B}, but not ET\textsubscript{A}, leads to salt-sensitive hypertension.\textsuperscript{51} In agreement, the effects of three doses of BQ-123 (0.1, 0.2, and 0.3 mg/kg) on renin hemodynamics, tubular function, and vasoactive hormones were measured in volunteers in a randomized, placebo-controlled, double-blind, dose-response study. The main effect was a dose-dependent increase in renal sodium excretion despite stimulation of the renin-angiotensin system as evidenced by an increase in angiotensin II levels, whereas there was little effect on atrial and brain natriuretic peptides or vasopressin.\textsuperscript{52}  
Goddard et al\textsuperscript{53} elegantly showed that ET\textsubscript{A} antagonism by BQ123 and angiotensin-converting enzyme (ACE) inhibition using enalapril were synergistic in reducing mean arterial pressure in volunteers. However, BQ-123 increased renal blood flow, increased urinary sodium excretion, and reduced renal vascular resistance only during ACE inhibition. These effects were abolished by ET\textsubscript{B} blockade using BQ788 and nitric oxide synthase inhibition, whereas cyclooxygenase inhibition had no effect. These results showed that synergism between ET\textsubscript{A} antagonism and ACE inhibition occurs via an ET\textsubscript{B}-mediated, nitric oxide–dependent, cyclooxygenase-independent mechanism. In patients with chronic kidney disease, TAK-044 beneficially reduced the mean arterial and systemic vascular resistance index and tended to increase renal plasma flow. TAK-044 had no effect on sodium or lithium clearance, or on the fractional excretion of sodium and lithium.\textsuperscript{54}  
Combining the results from a number of different studies has led to the proposal that antagonism of ET\textsubscript{B} may be undesirable in conditions such as chronic renal failure, and therefore ET\textsubscript{A}-selective antagonists might be superior to mixed ET\textsubscript{A}/ET\textsubscript{B} antagonists. This hypothesis was tested experimentally by comparing the action of BQ123 or BQ788 alone or in combination in hypertensive patients with chronic renal failure.\textsuperscript{55} Blocking the ET\textsubscript{A} alone significantly reduced blood pressure in these patients. The magnitude of change was significantly higher than when ET\textsubscript{B} also was blocked by BQ788. BQ788 alone caused the expected systemic and renal vasoconstriction, supporting the concept that ET\textsubscript{B} maintain tonic renal vasodilatation in patients. BQ-123 infused alone increased renal blood flow and renal vascular resistance and reduced proteinuria, consistent with a renoprotective action. This effect was lost when ET\textsubscript{B} were blocked by infusing both BQ788 and BQ123. There was no change in sodium excretion but this may
have been the result of a comparatively small number of subjects.\textsuperscript{55}

In a larger study of 22 patients with nondiabetic proteinuric chronic kidney disease, BQ-123 produced significant natriuresis, resulting from increased renal blood flow. In addition, ET\textsubscript{A} antagonism reduced blood pressure and proteinuria, and, a new finding, decreased arterial stiffness.\textsuperscript{56} However, in diabetic patients with chronic kidney disease, avosentan (ET\textsubscript{A}-selective nonpeptide antagonist) was reported to be detrimental as a result of fluid overload.\textsuperscript{57}

**ET-RECEPTOR BLOCKADE IN CHRONIC KIDNEY DISEASE**

Receptor antagonists have emerged as the only strategy in the clinic for blocking the unwanted actions of ET-1. To date, no alternative strategies, such as inhibitors of ET converting enzymes or combined endothelin-converting enzyme (ECE)/neutral endopeptidase (NEP) inhibitors, have been approved. Four compounds, bosentan, ambrisentan, sitaxentan, and macitentan, originally were approved for clinical use in pulmonary arterial hypertension (PAH) (Table 2).\textsuperscript{11} Sitaxentan, however, was withdrawn from clinical use in 2010\textsuperscript{58} after idiosyncratic hepatitis occurred resulting from acute liver failure, leading to death. PAH affects approximately 100,000 patients in the United States and Europe and currently there is no cure. The disease is characterized by constriction and remodeling of pulmonary vessels, with high blood pressure in the lungs. This leads to right heart failure, which is the ultimate cause of death. Interestingly, although ET\textsubscript{A} are increased significantly in the failing right ventricle of patients with PAH\textsuperscript{59} and the failing left ventricle of patients with heart failure,\textsuperscript{60} clinical trails have failed to show a benefit in patients from the latter group.\textsuperscript{12} The reasons for this are unclear, but the action of ET antagonists on the vasculature may be more important in restoring the imbalance between ET-induced constriction and opposing vasodilatation of blood vessels.

In theory, the selectivity of antagonists should have pharmacologic and pathophysiological consequences. Selectively blocking smooth muscle ET\textsubscript{A} would be expected to lead to vasodilatation and attenuate proliferation, migration, fibrosis, and hypertrophy. Endothelial ET\textsubscript{B}, particularly in kidney, lung, and liver, should continue to bind and remove ET-1 where it is overexpressed in pathophysiological conditions, as well as releasing vasodilators to mediate their antiproliferative and antithrombotic actions.

**HOW DO WE DEFINE ANTAGONIST-RECEPTOR SELECTIVITY?**

These four antagonists represent a spectrum of selectivity ranging from bosentan, which is classified by the

---

**Table 2. Structure and Pharmacokinetic Properties of ET-Receptor Antagonists in Clinical Use**

<table>
<thead>
<tr>
<th></th>
<th>Bosentan</th>
<th>Macitentan</th>
<th>Active metabolite of macitentan</th>
<th>Ambrisentan</th>
<th>Sitaxentan (withdrawn from clinical use in 2010)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Trade name</td>
<td>Tracleer (Actelion, Allschwil, Switzerland)</td>
<td>Opsumit (Actelion)</td>
<td></td>
<td></td>
<td>Thelin (Pfizer, Groton, Connecticut)</td>
</tr>
<tr>
<td>Other names</td>
<td>Ro47-0203</td>
<td>ACT-064992</td>
<td>ACT-132577 Sulfamide</td>
<td>LU-208075</td>
<td>TBC-11251 3-Thiophenesulfonamide</td>
</tr>
<tr>
<td>Chemical name</td>
<td>Benzencesulfonamide</td>
<td>Sulfamide</td>
<td></td>
<td>Benzenepranoic acid</td>
<td></td>
</tr>
<tr>
<td>Structure</td>
<td><img src="image" alt="Bosentan Structure" /></td>
<td><img src="image" alt="Macitentan Structure" /></td>
<td><img src="image" alt="Ambrisentan Structure" /></td>
<td><img src="image" alt="Sitaxentan Structure" /></td>
<td><img src="image" alt="Sitaxentan Structure" /></td>
</tr>
<tr>
<td>ET plasma levels after administration</td>
<td>↑↑</td>
<td>11</td>
<td>-</td>
<td>↑</td>
<td>↓</td>
</tr>
<tr>
<td>Bioavailability</td>
<td>~ 50%</td>
<td>Not reported</td>
<td>Not reported</td>
<td>High</td>
<td>70%-100%</td>
</tr>
<tr>
<td>Time to maximum plasma concentration</td>
<td>3-5</td>
<td>16</td>
<td>40.2-65.6</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Terminal half life (hours)</td>
<td>5.4</td>
<td>16</td>
<td>40.2-65.6</td>
<td>15</td>
<td>10</td>
</tr>
<tr>
<td>Excretion in urine (%)</td>
<td>&lt; 3</td>
<td>Not detected</td>
<td>Not detected</td>
<td>Low</td>
<td>50-60</td>
</tr>
</tbody>
</table>

---
pharmaceutical company Actelion (Allschwil, Switzerland) as a mixed or balanced ET\textsubscript{A}/ET\textsubscript{B} antagonist, to sitaxentan, the most ET\textsubscript{A} selective. No consensus has emerged about the relative merits of mixed versus ET\textsubscript{A}-selective compounds in PAH.\textsuperscript{61,62} Some animal studies have suggested that selective ET\textsubscript{A} antagonism that leaves ET\textsubscript{B} unopposed and unblocked is beneficial,\textsuperscript{63} whereas other studies have shown mixed and ET\textsubscript{A}-selective antagonists have similar outcomes. The advantage of animal studies are that compounds can be compared head to head, but given differences in cell expression of subtypes these may not necessarily be informative of clinical studies in human beings. No clear-cut advantage of one over another has been reported for selective versus nonselective antagonism in PAH. However, in chronic kidney disease, the function and distribution of receptors suggests an ET\textsubscript{A} antagonist would be preferable in blocking ET\textsubscript{A}-mediated constriction and proliferation but sparing endothelial cell vasodilatation, clearing ET from the plasma, and natriuresis.\textsuperscript{64}

The selectivity of a ligand for two receptors usually is calculated by measuring the equilibrium dissociation constant (K\textsubscript{D}) for the two subtypes, in this case ET\textsubscript{A} and ET\textsubscript{B}, to provide a ratio of selectivity\textsuperscript{65,66} in ligand-binding assays. There is no standardized method or general agreement among pharmaceutical companies to determine which compound should be classified as ET\textsubscript{A} selective versus a mixed antagonist.\textsuperscript{62} Accurate information is essential in interpreting results from experiments in animal models and clinical trials as to whether the doses used are likely to result in a compound occupying only ET\textsubscript{A} or both subtypes. We have proposed that ET\textsubscript{A}-selective compounds should have at least a 100-fold selectivity for the ET\textsubscript{A} subtype whereas mixed antagonists should have less than 100-fold ET\textsubscript{A} selectivity.\textsuperscript{65} The reason for this is that the degree of receptor occupancy achieved when an antagonist is administered in vivo or in vitro is proportional to the concentration and can be calculated from the affinity using the following formula: L*/\left(K\textsubscript{D} + L\right), where L* is the free ligand concentration and K\textsubscript{D} is the affinity constant. For example, a compound that has an affinity measured in a ligand-binding assay of 1 nmol/L for ET\textsubscript{A} but 100 nmol/L for ET\textsubscript{B} would have 100-fold selectivity for ET\textsubscript{A}. By using this equation, at a concentration of 10 nmol/L, 90% of ET\textsubscript{A} are calculated to be blocked but less than 10% of the ET\textsubscript{B}. Although this concentration can be achieved accurately under controlled in vitro conditions, 100-fold selectivity is likely to represent the minimum that can be used in vivo to achieve selective ET\textsubscript{A} blockade. If the plasma concentration of this antagonist was increased to 100 nmol/L, 50% of ET\textsubscript{B} then would be occupied. Compounds of greater than 1,000-fold selectivity are likely to be needed for clinical or in vivo studies to ensure ET\textsubscript{A} selectivity is maintained.

As proof of principle, the effect of selective blockade was measured using TAK-044, a peptide antagonist with approximately 250-fold selectivity for the ET\textsubscript{A} subtype over ET\textsubscript{B} as measured by ligand binding in the human heart. A 30-mg infusion over 15 minutes of TAK-044 (providing a serum concentration of 2 nmol/L, calculated to block >95% of ET\textsubscript{A} but <5% ET\textsubscript{B}) had no effect on the immunoreactive plasma concentrations of ET-1. However, after a higher dose of 750 mg TAK-044 (providing a serum concentration of 80 nmol/L, calculated to block >99% of ET\textsubscript{A} and >75% ET\textsubscript{B}), the immunoreactive plasma ET-1 concentrations were increased more than threefold over basal levels. Importantly, the concentrations of the ET-1 precursor or C-terminal fragment of big endothelin-1 were unchanged, indicating that the increase in ET-1 in the plasma was unlikely to be the result of increased synthesis or release. The most likely sources of endothelin contributing to the observed increase were displacement of receptor-bound peptide and a reduction in plasma clearance mediated by ET\textsubscript{B}.\textsuperscript{45}

**DOES SELECTIVITY MATTER IN CHRONIC KIDNEY DISEASE?**

Blocking ET\textsubscript{B} clearly results in a significant increase in circulating plasma ET-1 levels. However, with a mixed antagonist, this increase is unlikely to be important because the vasoconstrictor ET\textsubscript{A} also is blocked. Side effects including headache, nausea, and nasal congestion have, to a certain extent, been reported for ET\textsubscript{A}/ET\textsubscript{B} mixed antagonists and with ET\textsubscript{A}-selective compounds. For ET\textsubscript{A}-selective compound such as ambri-sentan, nasal congestion and peripheral edema are more prevalent but they have less of the hepatic effects such as an increase in liver enzyme levels that require liver function tests and drug–drug interactions that are associated with mixed antagonists such as bosentan.\textsuperscript{67} Studies in mice selectively knocking out ET\textsubscript{A} from the nephron or collecting duct did not show ET\textsubscript{A} antagonist-induced fluid retention and this was attenuated where ET\textsubscript{A} smooth muscle had been deleted, suggesting the mechanism is a direct action on collecting duct receptors and partially within the vasculature.\textsuperscript{68}

Liver toxicity has been a significant problem with bosentan but its mechanism of action has been proposed to be independent of ET receptors and is thought to occur by inhibiting the bile salt export pump leading to accumulation of cytotoxic bile salts, resulting in hepatocellular damage. In contrast, macitentan is thought to enter the liver via passive diffusion and not by active uptake.\textsuperscript{69} As a result, macitentan has been reported to have a better safety profile compared with
bosentan for hepatic toxicity. This is an important consideration in patients with renal or hepatic disease.\(^7^0\) Bosentan is a competitive antagonist of ET\(_A\) and ET\(_B\) of the sulfonamide class,\(^7^1\) with a comparatively short half-life and good bioavailability. Bosentan, as with other dual antagonists, tends to have lower rates of fluid retention and edema when used clinically. Although bosentan has been shown to be effective in animal models of renal disease, the compound has not been evaluated in detailed clinical trials involving renal patients. Ambrisentan represents the second chemical class,\(^7^2\) is less ET\(_A\) selective than sitaxentan, and has good bioavailability and a long half-life. However, again clinical studies have not been reported in chronic kidney disease. Key clinical studies have been performed using sitaxentan,\(^7^3\) the most ET\(_A\)-selective antagonist that largely supports the hypothesis of selective ET blockade. A randomized, double-blind, three-way, cross-over study of patients with proteinuric chronic kidney disease compared sitaxentan and nifedipine with placebo for proteinuria, blood pressure, and arterial stiffness. As expected, plasma levels of ET-1 were unchanged during sitaxentan treatment, indicating that ET-1 continued to be cleared from the circulation but urinary ET-1 levels were decreased. Blood pressure, arterial stiffness, and proteinuria also were reduced significantly over 6 weeks. Intriguingly, asymmetric dimethylarginine, an endogenous inhibitor of nitric oxide synthases that is considered an independent marker of disease progression, was increased in these patients, supporting the concept that sparing ET\(_B\) receptors from blockade with sitaxentan treatment would modulate the nitric oxide pathway. Importantly, these effects were seen in patients already receiving optimal treatment with ACE inhibitors and angiotensin blockers. A related study also found an increase in nocturnal dipping in blood pressure with sitaxentan.\(^7^4\) The results suggested that ET\(_A\) antagonism had additional longer-term renoprotective effects in patients with chronic kidney disease.\(^7^5\)

**ET ANTAGONIST FOR THE FUTURE: MACITENTAN AND ATRESENTAN**

Macitentan is an insurmountable antagonist, resulting from structure-activity studies to improve the efficacy and tolerability of bosentan, and gained approval in the United States in 2013 for the treatment of PAH. Actelion describes the compound as a dual antagonist, but on the basis of their own data measuring inhibition of \(^{[125]}\text{I}-\text{ET-1}\) binding to human-expressed receptors it displays approximately 800-fold selectivity for the ET\(_A\) subtype.\(^7^4\) However, plasma ET-1 concentrations were increased significantly (two-fold at the highest dose tested), suggesting blocking of ET\(_B\) occurs at the dose used.\(^7^5\) A metabolite of macitentan, ACT-132577, is active pharmacologically, albeit with a lower potency, but reaches higher plasma concentrations and has a longer half-life than macitentan.\(^7^6\)–\(^7^9\) Key pharmacologic parameters suggest macitentan will have the potential for greater efficacy and safety than bosentan. Macitentan has a much longer receptor occupancy (17 minutes compared with 70 seconds for bosentan), probably as a result of interaction with different amino acid residues in the ET receptors and is an order of magnitude more potent than bosentan, measured by in vitro assays. Pharmacokinetic benefits include fewer interactions with other drugs, with no requirement to alter doses in patients with renal (or hepatic) impairment. Crucially, the compound has improved hepatic safety and reduced edema/fluid retention compared with bosentan.\(^7^9\),\(^8^0\) A number of clinical trials are actively recruiting, however, these do not yet include chronic kidney disease patients.\(^8^1\)

Clinical trials also recently were reported on an investigational ETA-selective antagonist: atrasentan (ABT 627).\(^8^2\) The aim of the double-blind study performed in parallel at two centers was to determine whether albuminuria was reduced further when atrasentan was administered at two different doses, with inhibitors of the renin-angiotensin system, to patients with type 2 diabetic nephropathy. Atrasentan reduced albuminuria at both doses tested, and reduced blood pressure, cholesterol, and triglyceride levels, with unwanted side effects being more manageable at the lower dose. These promising results lead to the initiation of a phase 3 multicenter trial (Study Of Diabetic Nephropathy With Atrasentan\(^8^3\)) with 4,000 patients.

**PERSPECTIVES**

After more than 25 years since the discovery of ET, the peptide remains the most powerful and long-lasting constrictor of the human vasculature including the kidney described to date. In pathophysiological conditions, ET-1 contributes to vascular remodeling, proliferation of mesangial cells, and extracellular matrix production mainly through binding to ET\(_A\). Beneficial actions of ET-1 on sodium and water regulation are mainly ET\(_B\)-mediated. These findings suggest an ET\(_A\)-selective antagonist would have a therapeutic advantage over a mixed antagonist in renal disease, and indeed the small number of acute studies directly comparing the peptide antagonists BQ123 versus BQ788 suggest ET\(_A\) blockade, sparing ET\(_B\), may be beneficial. However, this is balanced by the possible greater prevalence of side effects such as edema reported for small-molecule, orally active ET\(_A\) antagonists compared with mixed antagonists, although the latter also have their limitations because of liver toxicity. In addition, head-to-head studies in patients
comparing orally active ET\textsubscript{A} antagonists with mixed antagonists have not been performed. The renal ET system remains a compelling target: will new therapies be clinically relevant in the future? This question may be answered by the next generation of ET antagonists.

REFERENCES


Endothelin-receptor antagonists