The endothelin system in pulmonary and renal vasculopathy: les liaisons dangereuses

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ABSTRACT: Endothelial cells regulate vascular tone largely by the actions of endothelin-1. Endothelin-1 is a potent vasoconstrictor, with effects that are dependent on the receptors to which it binds as well as their location. Endothelin-1 dysregulation is implicated in pathological conditions, including those of the pulmonary vasculature and the kidney. In this review, we describe the physiology and actions of endothelin-1 in lung and renal tissues and discuss therapies that disrupt these interactions in disease states. We provide an overview of the current clinical progress of these targeted agents and provide perspectives on the treatment of pulmonary and renal diseases with endothelin receptor antagonists.

KEYWORDS: Chronic kidney disease, endothelin, pulmonary hypertension
but contributes little to vasoconstriction in either normal or diseased vessels [7]. ETβ receptors are predominantly expressed on ECs throughout the human vasculature, including the lungs [13, 15]. Upon activation by ET-1, ETβ receptors mediate the beneficial release of endothelium-derived relaxing factors, such as NO, in a feedback mechanism that limits ETα vasoconstriction. High densities of ETβ receptors are present in the lung, kidney and liver, where this subtype functions as a clearing receptor to internalise the ligand-receptor complex and remove ET-1 from circulation [16–19]. As a result, the plasma half-life of ET-1 is comparatively short [20].

In marked contrast to vascular smooth muscle, ETβ receptors predominate in the human kidney, comprising ~70% of the receptors present in both the cortex and medulla [21, 22]. ETβ receptors are present on ECs throughout the renal vasculature, consistent with a role in mediating dilatation by endothelin-derived vasodilators as well as clearing receptors, thus, removing ET-1 from circulation (fig. 2). ETβ receptors are also present on epithelial cells throughout the tubular epithelium, particularly the inner medullary collecting duct where the major action of ET-1 is to promote diuresis and natriuresis (table 1).

The majority of the ETα receptors are localised to renal vessels of the medulla and cortex, including the main renal and arcuate arteries and adjacent veins at the cortico-medullary junction, as well as intra-renal resistance vessels and arterioles supplying the glomerulus [22]. ETα is also present on other cell types including mesangial cells and podocytes [21].

In addition to removing ET-1 from circulation, the human kidney is also a source of substantial amounts of ET-1, with ET-1 staining localised to the cytoplasm of ECs lining intra-renal vessel arteries, veins and resistance vessels, as well as ECs within the glomerulus [23]. Intra-renal ET-1 primarily acts in an autocrine or paracrine manner with its main physiological actions being ETA-mediated constriction in the vasculature and ETβ-mediated diuresis and natriuresis in the renal medulla. In renal disease, ET-1 causes vasoconstriction as well
as extracellular matrix production, mesangial-cell proliferation and inflammation, mainly via ETA receptors [21]. Interestingly, the renal vasculature is almost an order of magnitude more sensitive to ET-1 than any other vascular bed [24, 25].

**RATIONALE FOR ET-1 RECEPTOR BLOCKADE: INCREASE OF ET-1 EXPRESSION AND RECEPTOR SUBTYPES**

High levels of ET-1 are present in multiple disease states, making the ET pathway an attractive target for pharmacological intervention. Increased ET-1 activity is present in patients with pulmonary arterial hypertension (PAH). In PAH, the blood flow through the pulmonary arterial circulation is reduced, leading to increased pulmonary vascular resistance and ultimately to right heart failure [26]. The condition is ultimately fatal; the 1-yr mortality rate remains high at ~15% with modern therapy [27]. A key review of the pathology, classification and treatment of PAH has recently been published [28].

Plasma levels of ET-1 are increased in PAH and correlate with the severity of disease, as well as its prognosis [29, 30]. Explanted lungs of patients with PAH show increased ET-1 production [31], as do lung biopsy specimens of paediatric patients with PAH [32]. Patients with PAH have reduced clearance of ET-1 in the pulmonary vasculature [33]. Furthermore, binding sites for both types of ET-1 receptors are upregulated in the distal pulmonary arteries of patients with PAH [34].

The ET system has also been implicated in kidney disease [5]. Plasma ET-1 levels increase as renal function declines in chronic kidney disease (CKD) [35, 36] and may contribute to hypertension. Upregulated renal ET-1 production is also observed in CKD [37], reflected in higher urinary ET-1 excretion [36, 38]. Animal data suggest that in CKD, the renal vasculature may be more sensitive to the vasoconstrictor effects of ET-1 than normal kidneys [39].

**SELECTIVE (ETA) OR NONSELECTIVE (ETA/ETB) RECEPTOR BLOCKADE?**

The various functions of each ET-receptor subtype ensure maintenance of vascular tone and function. Because ETA receptors in the pulmonary system are associated with multiple deleterious effects in PAH [40], inhibition is crucial. Nonselective ET-1 receptor antagonists (classified as blocking ETA and ETB receptors with similar potency) have reversed some of the observed pathological conditions associated with diseases, which may include inhibiting some functions of the ETB receptors on vascular smooth muscle, whose role in disease is poorly understood. However, because in healthy tissue the endothelial ETB receptor opposes and clears ET-1, dual blockade may not be desirable. Furthermore, the elevated levels of ET-1 that may result from ETB receptor blockade may not be desirable in PAH, where ET-1 levels are already dysregulated.

In the kidney, there are several reasons why ETA-selective antagonists might be superior to mixed blockade [21]. The pathogenic actions of ET-1 in the kidney (including vasoconstriction, extracellular matrix production and inflammation) are largely mediated by ETA receptors. Inhibition of the renal medullary ET-1/ETB system causes sodium retention and hypertension because of its role in systemic fluid and
electrolyte homeostasis. Additionally, ETβ receptors counteract the vasoconstrictive effects of ETA receptors and are essential for removing ET-1 from the systemic circulation.

Therefore, nonselective antagonism would be expected to block the natriuretic, diuretic and vasodilatory effects of the ETβ receptors in the kidney and the antihypertensive effects of ETβ receptors in the circulatory system, in addition to opposing the detrimental effects of ETA receptors [21]. Additionally, reduced ETβ-mediated clearance would probably increase plasma ET-1 levels [21]. In agreement, preclinical data show that selective ETA antagonism may be preferential to nonselective blockade in CKD [41, 42].

Although the role of the ET system has been widely studied, little is known about the potential interactions between the renal and the pulmonary circulation. However, PAH patients have significant renal dysfunction, which is likely to adversely affect prognosis [43]. Additionally, renal crisis in the scleroderma population is, at least in part, mediated by the ET system. Thus, there is the potential for cross-talk between the pulmonary and kidney vasculature via the ETA and ETβ receptors [43].

CLINICAL EVIDENCE FOR ET-1 BLOCKADE IN PAH

ET-receptor antagonists have been studied most extensively in PAH; several compounds have been approved for the treatment of the disease. ET-receptor agonists have also been investigated for the treatment of cardiovascular disorders, systemic hypertension, and chronic and acute heart failure [40].

Several agents have been developed for clinical use and are classified as either nonselective (including bosentan and tezosentan), modestly ETA selective (including ambrisentan and darusentan) or highly ETA selective (sitaxentan). The distinction between antagonists that are ETA selective and those that block both receptors is not precise, but theoretically the former should display at least 100-fold selectivity for the ETA subtype so that concentrations can be achieved to block >90% of the ETA but <10% ETβ (fig. 3) [44]. In practice, it is difficult to achieve a steady-state concentration with precision, even in experimental clinical studies; selectivities >1,000-fold may be necessary and are currently only achieved with sitaxentan. Blocking the ETβ subtype increases the level of circulating plasma ET-1 [17], which can serve as a biomarker of ETβ receptor antagonism. In contrast with the experience in pulmonary hypertension, all clinical trials of heart failure have used mixed antagonists or doses of modestly selective ETA antagonists high enough to block the ETβ receptor (evidenced by a rise in plasma ET-1), with disappointing results [45].

Nonselective antagonists

As the first orally available, dual-receptor antagonist in its class, the clinical experience with bosentan is the most extensive (table 2). The first randomised, double-blind, placebo-controlled study of bosentan examined 32 patients with functional class III or IV idiopathic PAH or PAH associated with the scleroderma spectrum of diseases [46]. Exercise capacity, as assessed by the 6-min walk distance (6MWD), was significantly improved (by a mean of 70 m) compared with baseline after 12 weeks of bosentan treatment; the improvement with bosentan was significantly greater than that observed with placebo treatment. Haemodynamically, bosentan significantly improved cardiac index and reduced mean pulmonary arterial pressure (Ppa) and pulmonary vascular resistance (PVR). New York Heart Association/World Health Organization (NYHA/WHO) functional class was significantly improved in patients receiving bosentan treatment. An asymptomatic increase in hepatic aminotransferase was observed in two patients treated with bosentan; the number and nature of adverse events (AEs) did not differ between placebo and bosentan groups.

A larger, double-blind, placebo-controlled trial examined 213 patients with WHO functional class III or IV idiopathic PAH or PAH associated with connective tissue disease (PAH-CTD) who received 16 weeks of bosentan treatment [49] at two doses of 125 and 250 mg b.i.d. The improvement in 6MWD observed with bosentan treatment was significantly greater than the deterioration observed with placebo treatment. Time to clinical worsening was defined as time to death, lung transplantation,

| TABLE 1 | Response to endothelin (ET)-1 in the kidney by receptor subtype |
|---------------------------------|------------------|
| **ETA receptor**                | **ETβ receptor** |
| Afferent arteriolar constriction | Medullary vasodilation |
| Efferent arteriolar dilation     | Afferent arteriolar constriction |
| Mesangial cell contraction      | Efferent arteriolar dilation |
| Mesangial cell proliferation    | Natriuresis       |
| Extracellular matrix accumulation| Diuresis         |
| Interstitial fibrosis           | Acid excretion ↑ |

*: effect depends on ET-1 concentration. Higher doses: afferent + efferent constriction (ultrafiltration pressure ↓), contraction mesangial cell (glomerular ultrafiltration ↓) → glomerular filtration rate ↓. Lower doses: efferent > afferent constriction (ultrafiltration pressure ↑), contraction mesangial cell (glomerular ultrafiltration ↓) → glomerular filtration rate ↔.
hospitalisation for PAH, lack of clinical improvement or worsening leading to discontinuation, need for epoprostenol therapy or atrial septostomy. In this study, the risk of clinical worsening was significantly reduced with bosentan compared with placebo treatment [49]. Abnormal hepatic function, syncope and flushing were more common with bosentan treatment. Elevated transaminase levels were more frequently observed at the highest dose of bosentan (250 mg b.i.d.), leading to drug discontinuation in 2% versus 0% in the active and placebo arm, respectively.

More recently, the effects of bosentan were examined in a 26-week study of 168 patients with earlier, functional class II PAH of multiple causes (including PAH associated with anorexigen use and congenital heart disease (CHD)) [51]. Co-primary endpoints were change in 6MWD and PVR. PVR was significantly improved with bosentan treatment but 6MWD was not, perhaps owing to the higher baseline 6MWD (435 m) of these less impaired patients, which may have made improvement difficult to detect. This study strongly supports the need for early intervention because vascular remodelling might be more feasible before disease progression [51].

Long-term bosentan treatment appears beneficial for survival, although to determine this equivocally would require ethically unacceptable extended studies with a placebo comparator arm. Kaplan–Meier survival estimates were 96% at 1 yr and 89% at 2 yrs for 169 patients with WHO functional class III or IV idiopathic or heritable PAH or PAH-CTD who received first-line bosentan therapy and continued in open-label extension studies. At the end of 1 and 2 yrs, 85% and 70% of patients, respectively, were alive and on bosentan monotherapy; additional or alternative therapy for patients was permitted at the discretion of the treating physician [54]. Survival in patients with functional class III idiopathic PAH treated with bosentan (n=139) was compared with a historical control group treated with epoprostenol (n=346) [55]. Improvements in exercise capacity and haemodynamics observed at 4 months were maintained at 1 yr. Kaplan–Meier survival estimates after 1 and 2 yrs were 97% and 91%, respectively, for bosentan and 91% and 84%, respectively, for epoprostenol. Notably, the epoprostenol group had more severe disease, but survival estimates were similar in matched cohorts of 83 patients. Strikingly similar observations for exercise capacity and haemodynamics were noted in a retrospective analysis of 103 consecutive NYHA functional class III/IV patients with idiopathic PAH treated with bosentan at a single centre [56]. Survival at 1 and 2 yrs was 90% and 87%, respectively; however, 44% of patients required additional prostanooid treatment. Elevated liver enzymes were noted in all of the long-term bosentan trials.

Bosentan has effectively treated other forms of PAH as well; supporting the idea that ET is a pathogenically relevant mediator in PAH of various aetiologies. Although the pathogenesis and mechanism of development of PAH associated with HIV are uncertain, 16 patients with functional class III and IV PAH-HIV who enrolled in an uncontrolled, prospective, 16-week study showed improved exercise capacity, quality of life and haemodynamics with bosentan treatment. Control of HIV infection was unaffected by treatment [57]. Furthermore, in a longer term (29 ± 15 months)
analysis of 59 patients with functional class II through to IV PAH-HIV (including 12 patients from the original study) treated with first-line bosentan, exercise capacity remained stable while haemodynamics were further improved; in 10 patients, haemodynamics stabilised and improved to WHO functional class I [58]. 11 consecutive patients with NYHA functional class III and IV portopulmonary hypertension (PPHT) and Child A cirrhosis with stable liver function who were treated for >1 yr with bosentan improved 6MWD/exercise capacity with a significant increase in peak oxygen uptake [59]. PVR fell significantly while other haemodynamic measures showed improvement (including cardiac output, $P_{pa}$ right atrial pressure, mixed venous oxygen saturation and mean systemic artery pressure), although a significant worsening of arteriovenous oxygenation was observed [60]. A retrospective study of 31 patients with PPHT who received either iloprost (n=13) or bosentan (n=18) revealed statistically significant differences in survival rates with bosentan treatment (94%, 89% and 89% at 1, 2 and 3 yrs, respectively) compared with iloprost treatment (77%, 62% and 46% at 1, 2 and 3 yrs, respectively) [61].

**ETA-selective antagonists**

Clinical success has also been demonstrated in PAH using selective ETA receptor antagonists. Sitaxentan (research code TBC-11251) is up to 6,500 times more selective for the ETA than ETB receptors [62]. Two randomised, double-blind, placebo-controlled studies examined the efficacy and safety of short-term treatment (STRIDE-1 and -2). In STRIDE-1, 178 NYHA functional class II through to IV patients with idiopathic PAH, PAH-CTD or PAH-CHD received placebo, 100 mg sitaxentan q.d. or 300 mg sitaxentan q.d. for 12 weeks [52]. Both doses of sitaxentan significantly improved 6MWD, although the primary end-point, the change in peak volume of oxygen consumed, was not significantly improved with 100 mg sitaxentan. Functional class and haemodynamic measures (cardiac index and PVR) were significantly improved with sitaxentan treatment. Liver function abnormalities were reported for 300 mg of sitaxentan but not for 100 mg of sitaxentan. Other frequent AEs included headache, peripheral oedema, nausea, nasal congestion and dizziness. Increased international normalised ratio or prothrombin time was the most frequently reported laboratory AE. In the second trial, 247 patients with WHO functional class II through to IV idiopathic PAH, PAH-CTD or PAH-CHD received placebo, 50 mg sitaxentan q.d. or 100 mg sitaxentan q.d. for 18 weeks; an open-label bosentan arm was included [53]. The primary end-point of 6MWD was significantly improved in the 100 mg sitaxentan but not the 50 mg sitaxentan group and was also significantly improved in the bosentan group. Significant improvement in WHO functional class only occurred in the 100 mg sitaxentan group. Elevated hepatic transaminases occurred for all treatment groups, including placebo, at a comparable rate.

In the STRIDE-2 extension trial for sitaxentan, PAH patients who completed the double-blind trial continued to receive either sitaxentan (n=145) or bosentan (n=84) for 1 yr. At 1 yr, sitaxentan therapy showed significant benefit over bosentan with respect to discontinuation of monotherapy, clinical worsening, elevated hepatic transaminases and survival (96% versus 88%, respectively) [63, 64], which, according to the authors, was related to the ETA receptor selectivity of sitaxentan [64]. However, this study does not provide a true head-to-head comparison because patients randomised in the bosentan arm were receiving open-label bosentan. In addition, placebo patients were randomised after the double-blind phase of the trial to receive sitaxentan 100 mg q.d. or bosentan 125 mg b.i.d., creating a potential bias in the analysis. Long-term treatment with 100 mg sitaxentan is associated with a 76% survival rate at 3 yrs, with 60% of the patients remaining on monotherapy [65].

Interestingly, sitaxentan has been investigated as an alternative ET-receptor antagonist for patients discontinuing bosentan therapy [66]. In a randomised, double-blind study of 48 patients with idiopathic PAH, PAH-CTD or PAH-CHD who had discontinued bosentan for inadequate efficacy (n=35) or safety concerns (n=13), patients received 50 or 100 mg sitaxentan q.d. In patients who discontinued for efficacy reasons, 100 mg sitaxentan improved 6MWD in five (33%) out of 15 patients compared with two (10%) out of 20 patients treated with 50 mg sitaxentan. Similar results were seen for the Borg dyspnoea index and WHO functional class. Of 12 patients who discontinued bosentan because of hepatotoxicity, only one patient developed elevated liver enzymes (after 13 weeks of sitaxentan therapy).

Of 42 PAH-CTD patients from the STRIDE-1 study, data from those who received 100 or 300 mg sitaxentan (n=33) were pooled and compared with data from placebo-treated patients (n=9) in a post hoc subset analysis [67]. After 12 weeks of treatment, mean 6MWD was significantly improved with sitaxentan treatment compared with the deterioration observed in the placebo group; parallel improvements in quality of life and haemodynamics were also observed. In 41 patients who entered the blinded extension study and received either 100 or 300 mg sitaxentan q.d. for a median treatment duration of 26 weeks, the proportion of patients having functional class I or II disease was significantly greater at the end of the study compared with the start of active therapy. Elevated liver enzymes were recorded in two patients.

Ambrisentan is another selective ETA receptor agonist; reports of the affinity of binding to ETA versus ETB receptors are variable but range up to 4,000-fold [68, 69]. The results of two concurrent, double-blind, placebo-controlled studies that randomised 202 and 192 patients with PAH, respectively, to placebo or ambrisentan (5 or 10 mg in ARIES-1; 2.5 or 5 mg in ARIES-2) orally once daily for 12 weeks followed by a 48-week extension study were published together [48]. The primary end-point of 6MWD was significantly increased in all ambrisentan groups. Time to clinical worsening was improved in ARIES-2 but not in ARIES-1, the difference lying primarily in a higher rate of deterioration in the placebo group observed in ARIES-2. Statistical improvements in WHO functional class, quality-of-life scores (assessed by the Short Form (SF)-36) and Borg dyspnoea score were observed in both studies. Liver abnormalities were not observed with ambrisentan treatment. Peripheral oedema, headache and nasal congestion were commonly associated with ambrisentan treatment.

In a subset of 280 patients from the concurrent trials who completed 48 weeks of treatment with ambrisentan monotherapy, the improvement in 6MWD observed at 12 weeks...
appeared to be maintained at 48 weeks [48]. Results of long-term response to ambrisentan in 383 patients with PAH (including those randomised to placebo in the controlled trials who received ambrisentan in an extension trial) have been presented recently [70]. Kaplan–Meier survival was estimated to be 94% and 88% at 1 and 2 yrs, respectively, whereas time to clinical worsening was estimated to be 88% and 72%, respectively. Of these patients, the incidence of aminotransferase levels more than three times the upper limit of normal (ULN) was 3.4%, which compares favourably with the 2.3% rate of increase observed in the placebo group during the first 12-week period.

Because ambrisentan was thought to have a lower risk of liver abnormalities than alternative ET-1-receptor antagonists, perhaps owing to the fact that it is propanoic-acid based, it was studied in 36 patients who had previously discontinued bosentan (n=31), sitaxentan (n=2) or both (n=3) owing to liver function test abnormalities [71]. In this 12-week trial, no patients had drug-related aminotransferase levels more than three times the ULN that resulted in drug discontinuation, the incidence of which was the primary end-point, although one patient had a transient aminotransferase level more than three times the ULN that resolved following a temporary dose reduction. In 34 patients who continued treatment in an extension study for a median of 102 weeks, no additional patients reported aminotransferase levels more than three times the ULN, despite the fact that >50% of patients doubled their ambrisentan dose. Significantly improved 6MWD and Borg dyspnoea index compared with baseline was observed at 8 and 12 weeks. Furthermore, WHO functional class improved in 43% of patients, and significant improvements were observed in six out of eight domains of the SF-36 health survey and the composite physical health score.

**Antagonists combined with other therapies**

Combination trials of bosentan with prostanoids were based on improved exercise capacity and apparent safety in uncontrolled case series [72, 73] but have shown mixed results (table 3). Bosentan was combined with epoprostenol, a continuously infused prostaglandin, in a double-blind, placebo-controlled prospective study [74]; 33 patients with idiopathic or familial PAH or PAH-CTD who were receiving epoprostenol were randomised to 16-week bosentan or placebo treatment. The bosentan plus epoprostenol group showed nonsignificant improvements in all measured haemodynamic parameters, failing to meet the primary end-point of total pulmonary resistance. The bosentan plus epoprostenol group had four withdrawals, including two deaths due to cardiopulmonary failure, one clinical worsening event and one AE compared with one withdrawal in the placebo plus epoprostenol group for an AE.

Inhaled iloprost, a prostacyclin analogue, was similarly added to stable bosentan therapy in a 12-week, randomised, double-blind trial of 67 patients, of whom 63 (93%) had NYHA functional class III idiopathic and associated PAH [75]. Placebo or inhaled iloprost (5 μg) was added to stable bosentan monotherapy. The bosentan plus iloprost patients improved their 6MWD (p=0.051) compared with bosentan plus placebo patients. Additionally, significantly more patients in the bosentan plus iloprost group improved NYHA status by one class than in the bosentan plus placebo group. Bosentan plus iloprost similarly demonstrated superiority in time to clinical worsening and haemodynamics. However, the clinical benefit observed could be attributable to iloprost alone because there was not a treatment arm in which iloprost replaced bosentan treatment. Another 12-week randomised controlled trial examining the addition of inhaled iloprost to bosentan treatment in 40 patients with idiopathic PAH was terminated early because the primary end-point of the 6MWD was not met [60].

The phosphodiesterase type 5 inhibitor sildenafil has been added safely to bosentan treatment after clinical deterioration following initial response to monotherapy in uncontrolled trials [59, 76]; controlled trials are underway. In the pivotal trial of tadalafil for the treatment of PAH, 53% of patients were on bosentan background therapy [77]. In contrast to treatment-naïve patients, improvements in 6MWD and WHO functional class were not significantly different in patients on background bosentan treatment who received 40 mg tadalafil versus placebo. Haemodynamics were “uniformly better” in treatment-naïve patients as well, although a small sample size was noted. Importantly, the sequential nature of treatment may have affected response; concurrent initiation of the two drugs in naïve patients may prove efficacious in adequately designed future trials. A case series of three patients with idiopathic PAH who sustained symptomatic and haemodynamic improvement after receiving sitaxentan and tadalafil in combination following bosentan failure may indicate promise with an ETA-selective agent [78].

Other combination trials are ongoing or planned with different compounds (table 2). Conflicting results from recent combination trials raise some significant concerns. Many of the patients in combination trials were diagnosed >3 yrs before randomisation and were already receiving therapy. Although their disease is worsening, these patients are clearly selected with a lower risk of event because outcomes for “prevalent” patients are better than those for new (“incident”) patients [79]. However, the reason for this discrepancy is unclear, perhaps because of differences in initial treatment at nonspecialised centres, age, comorbidities or the prevalence of associated CTD.

Furthermore, the ideal strategy to treat disease is currently unclear. Are more aggressive approaches, such as initial combination therapy or quick escalation of therapy in response to unmet clinical goals, necessary for favourable long-term outcomes? More studies are necessary, yet study design has been extensively debated. A recent expert consensus document discussed the best tools to assess the effects of current or new compounds in PAH [28]. It was recommended that future trials use robust end-points, such as time to clinical worsening, to assess the efficacy of new treatment strategies.

**CLINICAL EVIDENCE FOR ET-1 BLOCKADE IN KIDNEY DISEASES**

In a randomised, double-blind crossover study of seven adult males with CKD (mean arterial pressure 103 mmHg, mean plasma creatinine 3.5 mg·dL⁻¹), the experimental cyclic hexapeptide ET-1-receptor antagonist TAK-044 was used to briefly block both ETA and ETB receptors [78]. Each patient received placebo and 100 mg and 750 mg TAK-044 as 15-min intravenous infusions, with treatments separated by ≥7 days. TAK-044 is
| First-line drug | Added therapy (type) | Current, complete and published studies | Subjects n | Duration | Primary end-point(s) | Significantly improved? | Other end-points |
|-----------------|----------------------|------------------------------------------|------------|----------|----------------------|------------------------|-----------------|
| **Bosentan**    | Inhaled iloprost (prostanoid) | [75] | 67 | 12 weeks | 6MWD, BDI, NYHA FC, TCW | No, yes, yes | Improved HD (\(\bar{P}_{pa}\), PVR) No change HD (others) |
| **Bosentan**    | Inhaled iloprost (prostanoid) | Terminated [55] | 40 | 12 weeks | 6MWD | No | |
| **Epoprostenol**| Bosentan (ERA) | [74] | 33 | 16 weeks | TPR | No | No change 6MWD, NYHA FC, HD (C, PVR, \(\bar{P}_{pa}\), \(\bar{P}_{ra}\)) |
| **Bosentan**    | Tadalafil (PDE5i) | [77] | 216 | 16 weeks | 6MWD | No (40 mg combination) | Improved WHO FC (40 mg combination) |
| **Bosentan or sildenafil** | Iloprost power 15 disc Recruiting PROWESS 15 (NCT00709956) | −63 | Single dose | 6MWD | NA | BDI |
| **Bosentan or sildenafil** | Inhaled treprostinil (prostanoid) Ongoing TRIUMPH (NCT00147199) | −235 | 12 weeks | 6MWD | NA | NYHA FC, BDI, QOL, TCW |
| **Approved ERA, PDE5i or both** | Oral treprostinil (prostanoid) Completed FREEDOM-C (NCT00325442) | −300 | 16 weeks | 6MWD | NA | BDI, TCW, dyspnoea-fatigue index, WHO FC |
| **Approved ERA, PDE5i or both** | Oral treprostinil (prostanoid) Terminated FREEDOM-DR (NCT00760916) | −170 | 12 weeks | 6MWD | NA | BDI, TCW, dyspnoea-fatigue index; WHO FC; trough 6MWD and BDI, NT-proBNP, HD |
| **Approved ERA, PDE5i or both** | Oral treprostinil (prostanoid) Recruiting FREEDOM-C2 (NCT00878978) | NR | 16 weeks | 6MWD | NA | WHO FC, NT-proBNP, QOL, biomarkers |
| **Approved PAH therapy** | Imatinib (tyrosine kinase inhibitor) Ongoing (NCT00477269) | −60 | 6 months | Safety | NA | WHO FC, BDI, HD, TCW, biomarkers |
| **ERA** | Riociguat (cGMP activator) Recruiting (NCT00810693) | −462 | 12 weeks | 6MWD | NA | PVR, NT-proBNP, WHO FC, TCW, BDI, EQ-5D |
| **Approved PDE5i** | Ambrisentan (ERA) Recruiting ATHENA-1 (NCT0017305) | −40 | 24 weeks | PVR (also \(P_{pa}\), \(P_{ra}\), CO as supportive) | NA | 6MWD, BDI, TCW, WHO FC, NT-proBNP, CAMPHOR QOL, survival |
| **Bosentan**    | Sildenafil (PDE5i) Recruiting (NCT00332395) | −106 | 12 weeks | 6MWD | NA | TCW, BDI, FC |
| **Bosentan**    | Sildenafil (PDE5i) | COMPASS-1 | −45 | Single dose | PVR | NA | TPR, NT-proBNP, acute vasoreactivity |
| **Sildenafil**  | Bosentan (ERA) Recruiting COMPASS-2 (NCT003349) | −250 | 16 weeks | Time to first morbidity/mortality event | | WHO FC, BDI, EQ-5D, 6MWD, TCW/Death, patient global self-assessment |
| **Bosentan**    | Sildenafil (PDE5i) Recruiting COMPASS-3 (NCT00433329) | −100 | 28 weeks | 6MWD (380 m) | NA | MRI parameters |
| **First-line combination** | Sildenafil + sildenafil (PDE5i) Recruiting (NCT00796668) | −180 | 12, 24 and 48-week assessments | TCW | NA | 6MWD, WHO FC, SF-36 |

PDE5i: phosphodiesterase type 5 inhibitor; cGMP: cyclic guanosine monophosphate; 6MWD: 6-min walk distance; BDI: Borg dyspnoea index; NYHA: New York Heart Association; FC: functional class; TCW: time to clinical worsening; TPR: total pulmonary resistance; PVR: pulmonary vascular resistance; \(\bar{P}_{pa}\): mean pulmonary arterial pressure; \(\bar{P}_{ra}\): mean right atrial pressure; CO: cardiac output; NA: not applicable; HD: haemodynamics; CI: cardiac index; WHO: World Health Organization; QOL: quality of life; NT-proBNP: N-terminal pro-brain natriuretic peptide; EQ-5D: EuroQol 5 Dimensions; CAMPHOR: Cambridge Pulmonary Hypertension Outcome Review; MRI: magnetic resonance imaging; SF-36: Short Form-36. #: the registered National Clinical Trial number is given in parentheses.
only modestly selective for the ETA receptor; the low concentration selectively blocks ETA but not ETB, whereas the higher concentration antagonises both ET-receptor subtypes.

Systemic and renal haemodynamics, as well as plasma immunoreactive ET-1, its precursor big ET-1 and the C-terminal fragment were measured before and after infusions [80]. No change in plasma ET-1 level was observed at the lower concentration of TAK-044. At the higher concentration, plasma ET-1 was increased two-fold without a concomitant increase in big ET-1 or the C-terminal fragment, implying that the increased plasma ET-1 in CKD does not result from de novo synthesis but rather reduced clearance of the peptide by ETB receptors.

Both doses of TAK-044 altered renal dynamics by lowering the effective filtration fraction [80]. Systemic vasodilatation and lowered blood pressure were observed at both doses of TAK-044, supporting the idea that ET-1 contributes to the maintenance of vascular tone in patients with CKD. Although the study was small, ET receptor antagonists may be beneficial for treating hypertension associated with CKD if this antihypertensive action is sustainable.

The first clinical study to assess ETA and ETB receptor antagonists in a head-to-head comparison used the experimental, but highly selective, peptide antagonists BQ-123 (ETA) and BQ-788 (ETB). In this randomised, double-blind, placebo-controlled, four-way crossover study, the antagonists were examined alone and in combination in eight hypertensive patients with chronic renal failure and eight matched healthy controls [42]. ETA receptor antagonism significantly reduced blood pressure in patients with chronic renal failure or without ETB receptor antagonism, but the magnitude of change was significantly higher in the BQ-123-only group. BQ-123 also increased renal blood flow (fig. 4) and renal vascular resistance and reduced proteinuria, consistent with a renoprotective action; these effects were not seen when BQ-123 was combined with BQ-788. Thus, ETB receptors maintain tonic renal vasodilatation. Consistent with this idea, ETB receptor blockade with BQ-788 produced both systemic and renal vasoconstriction in chronic renal failure patients and healthy controls. Surprisingly, no changes in sodium excretions were observed despite evidence from animal studies that ETB receptors mediate natriuresis [5]. Although these were acute studies, the results suggest an ETA selective antagonist would reduce blood pressure in patients with chronic renal failure and provide additional renoprotection. Additionally, this study suggests that therapeutic effects can be expected provided that the ETA receptors are blocked, regardless of ETB receptor blockade.

In a randomised, placebo-controlled, double-blind, dose-response study in 11 healthy males, BQ-123 was infused intravenously in three separate doses (0.1, 0.2 and 0.3 mg·kg\(^{-1}\) for 1 h) [81]. Renal haemodynamics, tubular function, vasoactive hormones (plasma concentrations of renin, angiotensin II, atrial and brain natriuretic peptides and vasopressin) and blood pressure were measured in healthy males. Renal sodium excretion was increased despite a decrease in diastolic blood pressure and stimulation of the renin–angiotensin system. This suggests the natriuretic effect of BQ-123 overrides the sodium-retaining effect of increased activity in the renin–angiotensin system.

In a larger double-blind, randomised crossover study in which 22 patients with non-diabetic proteinuric CKD received, on two separate occasions, BQ-123 or placebo [82], ETA receptor antagonism significantly reduced blood pressure, proteinuria and arterial stiffness (a key determinant of cardiovascular risk) measured by pulse wave velocity. Importantly, all patients were treated with angiotensin-converting enzyme (ACE) inhibitors and/or angiotensin receptor antagonists (ARAs) titrated to the maximum tolerated dose throughout the study, so these effects occurred in addition to standard treatment. The reductions in proteinuria and arterial stiffness were partly independent of blood pressure because a subset of 10 patients who received 10 mg nifedipine as a third active control treatment demonstrated only comparable reductions in blood pressure and renal flow. Interestingly, BQ-123 produced a significant natriuresis in these patients, probably resulting from increased renal blood flow with ETA-receptor antagonism.

As in the previous trial, this was an acute study in a relatively homogenous group of patients. Patients with diabetes mellitus, vasculitis and renal vascular disease may benefit similarly from treatment. If the effects of ETA receptor antagonism can be maintained for longer periods, such treatment may confer cardiovascular and renal benefits in patients with CKD. Longer term trials are required to address these questions.

**FUTURE PERSPECTIVES: A ROLE OF CARDIO-RENAL INTERACTIONS WITH SELECTIVE BLOCKADE?**

Acute experimental clinical studies show that selective blockade of the ETA receptor preserves the natriuretic, antihypertensive and ET-1 clearance effects of ETB receptors, whereas nonselective blockade may reduce these effects in patients with CKD. Furthermore, it may be beneficial to combine an ETA antagonist with ACE inhibitors or ARAs for CKD treatment. The sustained safety and efficacy of ET-receptor blockade observed in PAH support a rationale for long-term randomised trials with...
nonpeptide antagonists. Furthermore, this potential may include other renal diseases associated with elevated levels of ET-1 or renal vasocostriction, such as acute renal failure, hepatorenal syndrome and cyclosporine therapy or characterised by cell proliferation, such as chronic renal failure [5].

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