**Endothelins in cardiovascular biology and disease**

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**Abstract** | Cardiovascular disease is a major contributor to global morbidity and mortality, and is the common endpoint of many chronic diseases. The endothelins comprise three structurally similar 21-amino acid peptides. Endothelin-1 and -2 activate two G-protein coupled receptors – endothelin-A and endothelin-B – with equal affinity, whereas endothelin-3 has a lower affinity for the endothelin-A subtype. Endothelin-1 is the most potent vasoconstrictor in the human cardiovascular system with remarkably long-lasting actions. It contributes to vasoconstriction, vascular and cardiac hypertrophy and inflammation, and to the development and progression of cardiovascular disease. ET receptor antagonists have revolutionised the treatment of pulmonary arterial hypertension. Clinical trials continue to explore new applications, particularly in treatment-resistant hypertension, chronic kidney disease and patients receiving anti-angiogenic therapies. Translational studies have identified key roles for the endothelin isoforms and new therapeutic targets in development, fluid-electrolyte homeostasis, and cardiovascular and neuronal function. For the future, novel pharmacological strategies are emerging *via* small molecule epigenetic modulators, biologics such as endothelin-B monoclonal antibodies and the potential of signalling pathway biased agonists and antagonists.

**Key points**

* Endothelin-1 (ET-1) is the most potent endogenous vasoconstrictor and contributes to basal vascular tone as well as a number of diseases such as hypertension, chronic kidney disease (CKD), pulmonary arterial hypertension (PAH) and preeclampsia.
* A common non-coding gene sequence variant regulates ET-1 signalling and is linked to 5 common vascular conditions: coronary artery disease, hypertension, migraine, cervical artery dissection and fibromuscular hyperplasia.
* The effects of ET-1 are mediated via two G-protein coupled receptors, ETA and ETB. There now exist both selective ETA and dual ETA/B receptor antagonists (ERA) available for use in the clinic.
* A phase 3 study (PRECISION) will explore the use of a long-acting selective ETA antagonist in treatment-resistant hypertension.
* For many patients with PAH, ERA therapy is considered first line.
* ERAs effectively reduce blood pressure and proteinuria on top of standard care in patients with CKD and have potential in scleroderma renal crisis, in the transition from acute kidney injury to CKD and in patients with end-stage renal disease requiring dialysis.

**Introduction**

*Biology of the endothelin system*

Following the identification of a vasoconstrictor peptide in the culture media of bovine aortic endothelial cells1, Yanagisawa and colleagues2 moved rapidly to identify the structure of a 21-residue vasoconstrictor peptide, named endothelin (ET). They showed it to be an extremely potent vasoconstrictor, with uniquely long-lasting effects. Additionally, they used cloning and sequencing to show that what we now know as ET-1 is generated through unusual proteolytic processing to generate the mature peptide, from an inactive precursor, big ET, by the action of a putative ET converting enzyme (ECE), providing a potential route to inhibiting ET-1 generation and actions [**Figure 1**]. They also recognised that ET-1 might be important in regulating basal vascular tone, and that its actions might contribute to the pathogenesis of hypertension and vascular spasm.

Subsequently, three ET peptides (ET-1, ET-2 and ET-3) with two unique cysteine-cysteine cross-links, two ET converting enzymes (ECE-1 and ECE-2) and two mammalian G-protein coupled receptors (endothelin-A (ETA) and endothelin-B (ETB)) have been identified. ET-1 is the most abundant isoform in the human cardiovascular system and the focus of this article. Although generated in many organs, including the heart and kidneys, the major source of ET-1 is the vascular endothelium, where ET-1 is continuously generated in blood vessels to maintain vascular tone3 and blood pressure (BP)4. ET-1 is largely secreted abluminally, towards the vascular smooth muscle, with picomolar levels in blood largely reflecting its action as a local hormone with overspill into the circulation. Elevation of plasma ET-1 is a marker of ETB antagonism5, so where levels are raised in disease they may relate to increased production or reduced clearance.

The major sites of ETA receptor mRNA expression are the vasculature, heart, lung, ovary and uterus, whereas those for ETB receptors are brain and lung6 [**Figure 2**]. From a combination of knockout, overexpression and pharmacological studies in health and disease6, we now know that that the major vascular actions of ET-1 on the vascular smooth muscle ETA receptor are to promote vasoconstriction, hypertension, hypertrophy, fibrosis and inflammatory changes including atherosclerosis [**Tables 1 & 2**]. By contrast, the endothelial ETB receptor stimulates local nitric oxide (NO) production and clears ET-1 from the circulation. Importantly, ETB receptors in the collecting ducts of the kidney appear to play an important role in promoting natriuresis7. However, the situation is not straightforward as ETB receptors on vascular smooth muscle can promote vasoconstriction, and may play a more prominent role in conditions like systemic and pulmonary arterial hypertension (PAH), and also contribute to adrenal generation of aldosterone [**Tables 1 & 2**]. An interesting and important recent study8 shows that a common non-coding gene sequence variant in chromosome 6 regulates ET-1 signalling and is linked to 5 common vascular conditions: coronary artery disease, hypertension, migraine, cervical artery dissection and fibromuscular hyperplasia.

Early work led to creation of the mixed ETA/B antagonist bosentan, and additional medicinal chemistry9 has generated a range of other small-molecule ET receptor antagonists (ERAs), either selectively inhibiting actions at ETA receptors, or with actions against both ETA and ETB receptors [**Table 3**]. Alongside these developments, BQ123 (ETA antagonist) and BQ788 (ETB antagonist) were critical in teasing out the physiological and pathological role of ET-1 in cardiovascular disease. Based on the actions of ET-1, early clinical research programmes with ERAs focused on hypertension, heart failure, subarachnoid haemorrhage and PAH, the last of these resulting in an orphan indication for bosentan, and subsequently ambrisentan and macitentan. Other research has focused on the actions of ET-1 in kidney disease, pain syndromes and sickle cell disease.

Development of ECE inhibitors has been disappointing and, even when combined as dual inhibitors of ECE and neutral endopeptidase (NEP), have not made it further than preliminary studies in the clinic. An alternative approach, suggested by the sulphonamide scaffold9, has generated sparsentan, the first and only current agent combining ERA and angiotensin receptor blocking (ARB) properties. Opportunities exist to develop further ERAs with non-sulphonamide structures, or by exploiting biased signalling, both of which might limit adverse effects.

*Adverse effects of ERAs*

On the basis of what is known from animal studies about the developmental role of ET-1, ERAs will be first-trimester teratogens. These agents are, therefore, used in women of reproductive age only when conception can be prevented. Based on on-target effects of ET-1, ERAs cause vasodilator effects such as flushing, headache and hypotension/faintness. They also cause as reversible reduction of blood haemoglobin, and cause fluid retention and dependent oedema, most likely though plasma expansion and altered Starling’s capillary forces. Sulphonamide structures, as with bosentan, are known to cause hepatic transaminitis and sitaxentan was withdrawn after it was found to cause severe liver failure. However, this is not a uniform problem, because liver dysfunction is not seen, for instance, with ambrisentan, a propionic acid derivative, and atrasentan, another agent without a sulphonamide scaffold9. These issues are borne-out by a meta-analysis10 that identifies hepatic transaminitis, peripheral oedema and anaemia as the main adverse effects of treatment with ERAs. Perhaps the most clinically challenging ongoing problem with the use of ERAs is fluid retention, which in hypertension has proved manageable with additional diuretic use or up-titration.

**Hypertension**

Yanagisawa2 first identified the long-lasting vasoconstrictor and pressor actions of ET-1 and predicted that it might be important in control of systemic BP and contribute to the pathogenesis of hypertension. This was followed by evidence that vascular production of ET-1 is increased in many (salt-sensitive) models of hypertension11 and that BP could be lowered by ET blocking agents in these models12. In people with hypertension, there is some evidence that resistance vessels in hypertensive subjects may be particularly sensitive to ERAs, and that plasma ET-1 levels are higher in people with hypertension, but these data are inconsistent6.

Given the global primacy of hypertension as a risk factor for cardiovascular disease13, it is perhaps not surprising that this was one of the first targets addressed with the dual antagonist bosentan14. Indeed, in a randomised controlled trial in patients with primary hypertension and a diastolic BP between 95 and 115mmHg, bosentan (100-2000mg once daily) was shown to reduce clinic BP by 6mmHg compared with placebo, similar to the effect of the ACE inhibitor enalapril (20mg once daily). However, there were some issues with liver dysfunction and fluid retention at these doses, influencing the balance of benefit to harms, and bosentan was subsequently successfully developed at lower doses for PAH. These encouraging observations were confirmed with the modestly ETA selective antagonist, darusentan (10, 30 and 100mg daily) which reduced BP by around 10mmHg at the highest dose and sustained the reduction after 6 weeks of treatment15.

Given the range of established treatments for uncomplicated primary hypertension, the focus has moved to treatment-resistant hypertension (TRH), in which BP is uncontrolled despite taking three or more agents of different classes at optimal doses, including a diuretic. This condition is estimated to affect ~10 million people globally. Importantly, this is a high-risk group of patients, often with comorbidity, and a poor outcome16. Early data suggest a role for the ET system in TRH17. On this basis, darusentan was studied in two major placebo-controlled randomised controlled trials. The first (DORADO)18, published in 2009, was undertaken in 379 patients with TRH, and showed that darusentan (50, 100, and 300mg daily), reduced the coprimary endpoints of seated systolic and diastolic BP at week 14 of treatment by 17/10, 18/10, and 18/11mmHg with increasing doses of darusentan, but significantly less than placebo at only 9/5 mmHg. The benefits were seen in all groups of patients, including those with type 2 diabetes and/or chronic kidney disease (CKD). The only major adverse effect of darusentan was fluid retention, occurring in ~25% of patients on darusentan (but only ~14% on placebo). However, these results were promising, and fluid retention seemed to be manageable by increasing the dose of diuretic without withdrawing patients from the study. A further study (DORADO-AC)19, this time with an active control, was undertaken for licensing purposes in 1,453 patients with TRH. The doses used were as in DORADO, as was the duration of treatment and the coprimary end point. The active control (guanfacine 1mg daily) was an unusual choice and unfamiliar to most physicians in this indication. Seated BP at week 14 was reduced by 15/10 mmHg with darusentan, significantly more than by guanfacine (12/6 mmHg) for both systolic and diastolic BP. However, placebo treatment unexpectedly reduced BP by 14/8 mmHg at 14 weeks. This response was not different from that of darusentan for systolic BP and greater than that of guanfacine for diastolic BP. Although darusentan was well tolerated, and outperformed both placebo and guanfacine, in a *post hoc* time-weighted analysis, and based on ambulatory BP monitoring (challenging the wisdom of using clinic BP measurements in key hypertension trials20), darusentan did not meet its prespecified coprimary endpoints, and this agent has not been further developed in TRH.

Recently, a UK study in TRH21 showed the clear superiority of the mineralocorticoid receptor antagonist, spironolactone (25-50mg daily), over the alpha-blocker doxazosin (4-8mg daily) and beta-blocker bisoprolol (5-10mg daily). Although used off-label, the evidence in support of spironolactone is strong. However, spironolactone may cause hyperkalaemia, and gynecomastia can be a problem in men, so there is still a need for an effective, well-tolerated and licensed treatment for TRH.

In this regard, Idorsia have recently announced the encouraging results of a phase 2 study with aprocitentan, the active metabolite of the ERA, macitentan22. Here, 490 patients were randomised to receive either aprocitentan 5, 10, 25, 50mg, placebo, or lisinopril 20mg once daily in patients with primary hypertension. As reported by Idorsia23, the study showed (in a per protocol analysis) a dose-dependent diastolic BP reduction of 6-12mmHg, compared with 5 and 8mmHg for placebo and lisinopril, respectively. Systolic BP reductions ranged from 10 to 18.5mmHg, and were 8 and 13mmHg for placebo and lisinopril. These findings were confirmed in an intention-to-treat analysis and by 24 hours ambulatory BP monitoring. As a result of these encouraging data, Idorsia has launched a phase 3 trial, the PRECISION study in TRH24, providing some optimism that a licensed agent for the treatment of TRH may emerge from our understanding of ET biology. In the treatment of a chronic asymptomatic risk factor for cardiovascular disease, like hypertension, poor adherence to treatment is a significant problem. In this case, agents with a half-life of 12 hours or more (see Table 3) have the benefit that they only need to be taken once daily. Aprocitentan has a particularly long half-life, which can also compensate for poor adherence associated with the occasional missed dose.

There are unresolved issues around whether the reduction in blood haemoglobin that is seen with ERAs might be an issue (there is no evidence of this as yet). It is also unclear how frequent fluid retention will be, and how easy to manage in TRH, where patients are already taking diuretics (though fluid retention was uncommon in the phase 2 study with aprocitentan). ETA receptor selectivity does not appear to be a clear benefit, as even very selective agents can cause fluid retention25, and there is no evidence that fluid retention is more frequent with dual antagonists. Research, though from the manufacturers of aprocitentan even suggests dual ERAs may be protective26. PRECISION may also provide an opportunity to explore whether there are biomarkers, such as plasma ET-1 or gene variants8, that select those patients most likely to respond.

**Heart failure**

Chronic heart failure (CHF) was one of the earliest indications explored for ERAs. This approach was underpinned by compelling preclinical studies showing increased myocardial expression of ET-1 in experimental CHF and improvement in myocardial function and survival with BQ-123 treatment in rats27,28. In human studies, investigators showed high plasma ET-1 in CHF, higher levels being associated with more severe disease. Short term intravenous administration of bosentan29 and BQ-12330 reduced systemic and pulmonary vascular resistance, and BP, while cardiac index increased. These promising effects were not associated with changes in heart rate, and only one case of symptomatic hypotension. These encouraging data prompted a number of companies to pursue CHF programmes with ERAs and four large, multicentre randomised controlled trials were initiated. Unfortunately, none was successful31. REACH-1 with bosentan discontinued early, because of elevated liver enzymes, and was replaced by ENABLE using a lower dose. Neither improved outcomes, and in both there was an excess of adverse effects in the active treatment arm. Enrasentan in the ENCOR study, and darusentan in the EARTH study were similarly unsuccessful. Only the EARTH study was fully published32 so we have been able to learn only limited lessons from this large body of work, a situation which is clearly disappointing, given the effort that went into running the studies, together with the commitment from the patients enrolled31. We anticipate in the current era that we would have sight of this information, both protecting patients and indicating potential ways forward. More recently, a trial was undertaken with bosentan in a subgroup of patients with CHF and secondary pulmonary hypertension, which addressed a key unanswered question but did not affect haemodynamic variables and was associated with more frequent adverse effects, requiring drug discontinuation33. These studies were all in patients with CHF and reduced ejection fraction (HFrEF), but there is one study with an ETA selective agent in patients with a preserved ejection fraction (EF >50%; HFpEF). This blinded randomised controlled trial compared sitaxentan (100mg daily) in 192 patients allocated 2:1 against placebo over 24 weeks34 and showed that active treatment significantly increased exercise tolerance, with no more adverse effects than placebo, but did not improve secondary endpoints such as left ventricular mass or diastolic function.

The story in acute heart failure (AHF) has been similarly disappointing. Initial small studies with a range of ERAs35 suggested a haemodynamic benefit and resulted in the RITZ programme of studies with tezosentan. RITZ-1 and RITZ-2 focused on early symptoms and haemodynamics, respectively, whereas RITZ-436 and RITZ-533 focused on effectiveness in patients with acute coronary syndromes and pulmonary oedema. In each case the study failed to show benefit except, perhaps unsurprisingly, RITZ-2 confirming the haemodynamic changes. These trials were followed by two identical large trials (VERITAS) undertaken in a total of over 1,400 patients with two of: *(1)* elevated plasma concentrations of B-type or N-terminal pro–B-type natriuretic peptide, *(2)* clinical pulmonary oedema, *(3)* radiologic pulmonary congestion or oedema, or *(4)* left ventricular systolic dysfunction37. In this study which compared tezosentan (5mg/h for 30 minutes, followed by 1mg/h for 24 to 72 hours [n=730]) to matched placebo [n=718] there was no improvement in symptoms of dyspnoea, or in deaths or worsening heart failure.

The development of ERAs in heart failure has provided some lessons, not least the disconnect (also found for other drug classes) between the haemodynamic ‘improvements’ observed in phase 1 & 2 studies, and the patient-based outcomes affecting symptoms of dyspnoea, or hospitalisations and deaths, in phase 338. Other key issues that have arisen are the high incidence of hypotension and acute kidney injury (AKI), linked to systemic vasodilatation, and the liver enzyme changes associated with some of the first-generation ERAs. In addition, it remains unclear whether lower drug doses might improve the balance of benefit to harm39. It seems perhaps unlikely that there will be further clinical trials with ERAs in either AHF or CHF, given the lack of clinical benefit, and possible harm of fluid retention (though this has been effectively mitigated with diuretic in the hypertension trials). Nevertheless, it is worth reflecting that an opportunity was missed to gain valuable information from the early unpublished CHF studies, that there have been no outcome studies undertaken in HFrEF with highly selective ETA antagonists (with BQ-123 used in some of the animal and proof-of-principle human studies), and that HFpEF remains a potentially promising avenue.

***Pulmonary arterial hypertension***

PAH mostly affects young women and untreated, most patients die within 2 to 3 years of diagnosis40. A sustained rise in pulmonary arterial pressure, due to increases in pulmonary vascular resistance (PVR), leads to dyspnoea, fatigue, chest pain and syncope. Plasma ET-1 is increased in PAH models and patients with PAH. This increase correlates with markers of severity, such as raised PVR and right atrial pressure, and poor outcomes correlates41. The source of this increased ET-1 is unclear and may either be a reflection of enhanced production42 or reduced ETB receptor-mediated clearance43. Since their introduction almost two decades ago, dual and selective ERAs have emerged as important treatments for PAH44. It is likely that ETA receptor-mediated effects contribute to disease progression and so their antagonism may be essential in treating the disease. The role of the ETB receptor in PAH is less clear. Whilst some transgenic and antagonist studies suggest a net vasodilatory (and so protective) role for the pulmonary ETB receptor in PAH45-48, others support a vasoconstrictor role for pulmonary ETB49. Thus, combined ETA and ETB blockade may be required for the complete inhibition of ET-1-induced vasoconstriction in PAH. Interestingly, recent clinical data suggest that ETA selective agents are more effective in combination with a phosphodiesterase-5 inhibitor than either drug alone50.This has been attributed to ET-1 activating endothelial ETB to release nitric oxide, the effect of which is enhanced by phosphodiesterase-5 inhibition and supports the use of selective ERAs in PAH.

Currently there are three ERAs licensed for the treatment of PAH: the mixed ETA/B ERA bosentanand the ETA selective ERAs macitentan and ambrisentan. These have been investigated in a number of clinical trials51. Bosentan (given at a dose of 125mg twice daily) improves symptoms, exercise tolerance, functional status and time to clinical worsening in patients with PAH52, even in those with mild symptoms53. However, there are no data supporting a mortality benefit. The SERAPHIN study, which explored the efficacy of macitentan, was a landmark study in PAH as, unlike previous studies, it enrolled a large number of patients (n=742), had over a 2-year treatment period, and had a composite primary endpoint of morbidity and mortality (as opposed to the standard 6-minute walking distance). Macitentan reduced the primary endpoint although this was due to a reduction in worsening of PAH; there was no reduction in all-cause or PAH-related mortality54. Ambrisentan was assessed in the ARIES studies and, similar to bosentan, improved symptoms, functional status and quality of life compared to placebo55. Overall, for many patients with PAH, ERA therapy is considered first line.

With respect to the clinical studies with ERAs in PAH there are no data on fibrosis. Animal studies show that overexpression of ET-1 leads to inflammation and fibrosis, especially in the lung56, and these effects are mediated by both ETA and ETB receptors57. However, transgenic studies support a protective role for the ETB receptor58 and a direct comparison of selective ETA, ETB and dual ETA/B receptor antagonism showed that only dual and selective ETA antagonism prevented pressure elevation and structural abnormalities59. Notably, mixed receptor blockade provided no additional benefit to selective ETA antagonism, while isolated ETB blockade aggravated pathological changes. Thus, in PAH, whereas blockade of both ETA and ETB receptors may be necessary in terms of haemodynamics (because of the upregulation of pulmonary vasoconstrictor ETB receptors), the structural changes are predominantly ETA receptor driven, and so its antagonism would appear necessary, with little added benefit, but no harm, associated with additional ETB receptor blockade. Currently, there are no studies that address this directly.

**Kidney disease**

*Proteinuric CKD*

ET-1 plays and important role in renal physiology and pathophysiology [**Figure 3**]. Proteinuria is a common feature of CKD and the degree of proteinuria is closely associated with renal and cardiovascular outcomes60. To date, there have been a number of acute and chronic dosing studies using both selective ETA and dual ETA/B receptor blocking approaches in diabetic and non-diabetic proteinuric CKD61-67. In summary, ERAs reduce proteinuria, an effect that is probably in large part mediated by the systemic and renal haemodynamic effects of these drugs. Additionally, ERAs improve circulating lipids and arterial stiffness in CKD68. Importantly, these effects manifest on top of standard care with blockers of the renin-angiotensin system.

The pivotal SONAR study69, a randomised, double-blind, placebo-controlled study using the ETA selective ERA atrasentan, aimed to recruit >4,000 patients with type 2 diabetes and proteinuria already on maximally tolerated doses of inhibitors of the renin-angiotensin system. The primary outcome was time to doubling of serum creatinine or occurrence of end-stage renal disease (ESRD). Unfortunately, this study was stopped prematurely as there were fewer endpoints than anticipated, but not due to concerns over safety. This may be a problem for future studies in diabetic CKD, where the benefits of sodium glucose cotransporter 2 (SGLT2) inhibitors have been shown for both cardiovascular and renal endpoints70. Publication of the SONAR study is eagerly awaited as although it was stopped prematurely it may show benefits of ERAs in CKD.

There have been encouraging results from the recently published DUET study which examined the safety and efficacy of sparsentan, a dual antagonist of the ETA and angiotensin II type I receptor in patients with primary focal and segmental glomerulosclerosis (FSGS)71, a condition with few therapeutic options. Sparsentan reduced proteinuria to a greater extent than an ARB alone and was well tolerated. These positive results are being taken forward into a larger phase 3 outcome study in FSGS72. Retrophin, the company that produces sparsentan, is also planning a study exploring its efficacy in immunoglobulin-A (IgA) nephropathy, the commonest glomerulonephritis worldwide73.

Although most studies have focussed on using ERAs in kidney disease, a small study in patients with type 2 diabetes and nephropathy examined the efficacy of the combined ECE and NEP inhibitor, daglutril74. In this 8-week, double-blind, placebo-controlled, crossover study, patients were optimised on standard therapy with an ARB. Daglutril failed to reach the primary endpoint, which was a reduction in albuminuria. However, there was a reduction in ambulatory BP with the most profound drop in night-time BP (systolic, diastolic and mean). There are potential problems with this pharmacological approach. Whereas ECE inhibition will block ET-1 production, NEP inhibition will decrease its degradation75. There are no further studies currently planned using this agent.

## *Ischaemia-reperfusion injury (IRI)*

AKI is responsible for ~2 million deaths annually worldwide and this number is increasing76. IRI is a leading cause of AKI77. Importantly, AKI is independently associated with an increased cardiovascular risk78, and many of those with AKI progress to CKD and even ESRD79, which will increase this risk further. Potential mechanisms explaining AKI transitioning to CKD, and indeed CKD progression, include systemic and renal hypertension, and glomerular and tubular hypertrophy, all leading to renal fibrosis. ET-1 may contribute to all of these processes and so targeting this system seems a plausible treatment for renal IRI80.

Current data support a role for ET-1 (acting through ETA)in AKI with upregulation of the system in relevant animal models81,82. Additionally, selective ETA antagonism given prior to injury prevents many of the short-term effects of injury83-86. However, no study has examined ERA administration in a therapeutic manner (after the injury) and over a longer period of time to assess the impact on disease progression. There also appears to be a protective role for ETB following IRI. Data suggest ETB downregulation and inactivation early after IRI and prevention of these by a mineralocorticoid receptor antagonist (MRA), which not only preserved renal function but also improved renal injury87. Interestingly, this protection was lost when there was concomitant dosing with a selective ETB antagonist. This may be due to a loss of ETB-mediated vasodilatation. Therefore, a decline in ETB expression, or indeed its inactivation, after IRI may lead to sustained vasoconstriction prolonging the ischaemic period. This might explain why ETA antagonism is unsuccessful in preventing the initial injury after IRI, a time when ETB appear to play a more crucial part.

These observations should inform future clinical trials in renal IRI both in terms of timing and choice of therapeutic agent. Preserving ETB receptor expression and function with a MRA might be beneficial as a preventive therapy in situations where IRI is highly likely, such as following cardiothoracic surgery. Alternatively, selective ETA ERAs may be more favourable for patients with existing AKI to prevent further decline and progression to CKD.

## *End-stage renal disease*

Cardiovascular mortality is highest amongst patients with ESRD on maintenance haemodialysis88. These patients have up to 10-fold increases in plasma and tissue ET-1, higher than in any other disease state89. This most likely represents a combination of increased production and reduced clearance of ET-1. An upregulated ET system may contribute to many of the common complications of dialysis such as systemic and pulmonary hypertension, ischaemic heart disease, heart failure, peripheral vascular disease, and stroke. In models of CKD, a selective ETA approach also reduces medial vascular calcification90, a major pathological feature of CKD. Vascular calcification promotes arterial stiffening and this is an independent risk factor for cardiovascular mortality in ESRD patients91. Although arterial stiffness can be reduced by selective ETA receptor antagonism in pre-dialysis CKD62 there remain no such data in ESRD. These sorts of studies could potentially offer a novel therapy in a group where few therapeutic interventions have been shown to improve outcome.

For some patients with ESRD, kidney transplantation is an alternative option to dialysis. Whilst a transplant may improve quality of life, it can be associated with both short and longer-term complications. These include calcineurin inhibitor (CNI) toxicity and IRI in the early post-transplant period, and hypertension and cardiovascular disease longer-term. CNI toxicity and IRI may lead to AKI with delayed graft function. This may progress to CKD; IRI is also associated with poor graft survival and increased risk of rejection92. A role for the ET system in the complications associated with kidney transplantation has been previously reviewed and the case for clinical trials in this area highlighted93.

*Scleroderma renal crisis*

Scleroderma is an autoimmune connective tissue condition characterised by chronic inflammation and fibrosis94. ERAs are licensed for the treatment of digital ulceration in this condition. Renal involvement, secondary to vascular damage and reduced renal blood flow, is common95. Scleroderma renal crisis, characterised by accelerated arterial hypertension with a rapid decline in renal function often requiring dialysis, is a relatively common and life-threatening complication. Scleroderma patients exhibit raised plasma and tissue concentrations of ET-196.

The ZEBRA study97 recently completed recruitment. This study explored the safety and efficacy of 1-year dosing with zibotentan (the most selective ETA antagonist available with no demonstrable ETB binding affinity98) in 72 patients with scleroderma renal crisis. The primary outcome was plasma soluble vascular cell adhesion molecule 1 (sVCAM 1), a measure of renal involvement in scleroderma. Its results are eagerly awaited.

**Subarachnoid haemorrhage**

In health, ET-1 does not contribute to cerebral vascular tone99. However, there is upregulation of the ET system (both the peptide and its receptors) following cerebral ischaemia and this may contribute to vascular dysfunction and brain injury100,101. This is the case in subarachnoid haemorrhage (SAH) where, from a clinical perspective, cerebral vasospasm is the only medically treatable cause of disability and death.

Pre-clinical data in models of SAH suggested potential benefits of ETA selective ERAs. However, early clinical trials used mixed ERAs were inconclusive102. Later studies with the selective ETA receptor antagonist clazosentan suggested that it prevented cerebral vasospasm following SAH, although the clinical outcome remained unchanged103. A meta-analysis published in 2012, which included data from 5 studies incorporating 2,601 patients, concluded that ERAs do not affect the functional outcome after SAH even though they reduce vasospasm104. This was no different for selective or dual antagonists.

At present, ERAs do not have a role in the medical management of SAH but the results of ongoing studies, perhaps in more defined cohorts using drugs with an improved side effect profile, are awaited105. Indeed, Idorsia recently announced a phase 3 registration study (REACT) assessing clazosentan for the prevention of clinical deterioration due to vasospasm-related delayed cerebral ischaemia following SAH106.

**Antiangiogenic therapies & preeclampsia**

Inhibition of vascular endothelial growth factor (VEGF) limits angiogenesis and is a therapeutic strategy in many cancers. Preeclampsia is characterised by an excess of the circulating soluble extramembranous part of the VEGF type-1 receptor (soluble fms-like tyrosine kinase-1, sFlt-1) which acts to sequester VEGF leading to its functional deficiency. Both are associated with an activation of the ET system107.

Antiangiogenic treatment is associated with the development of hypertension and proteinuria108 with a thrombotic microangiopathy evident on renal biopsy109. These side effects, seen in ~30% of those taking these agents, can be treatment limiting. Animal and human studies have shown a rise in plasma ET-1 following VEGF inhibition using a number of agents suggesting this is a class effect110,111. A rise in circulating ET-1 is also a consistent feature in women presenting with preeclampsia and associated with the rise in sFlt-1112. In models of preeclampsia, there is a rise in BP with the development of proteinuria; in respect of the ET system there is an increase in plasma ET-1 with increased expression of preproET-1 and the ETA receptor in both the kidney and uterus113. Based on antagonist studies (using both selective and mixed ERAs), it is clear that ET-1 plays an important direct role in the pathogenesis of the hypertension and proteinuria seen in preeclampsia114,115 and following VEGF inhibition116,117.

To date, there have been no clinical studies exploring the utility of ERAs in either the setting of VEGF inhibition or preeclampsia. The potential use of ERAs alongside VEGF inhibition is an attractive option. This might not only allow the continuation of disease-modifying therapy but also slow tumour progression as dysregulation of the ET system is now recognised to play an important role in the biology of several solid tumours, including breast, colon, lung, and ovarian cancers118 all of which have VEGF inhibition as a treatment option. There is one ongoing study (ENDEAVOUR) exploring the use of ERAs in VEGF inhibitor-triggered hypertension and its results are eagerly awaited119. ERAs are teratogenic and so their use in pregnancy, at least in its early part, is prohibited. Emerging data suggest that preeclampsia is associated with a long-term risk of cardiovascular disease120 and whether ERAs, administered late in pregnancy or in the post-partum period might reduce this risk, at a time when teratogenic effects should not apply, remains unexplored.

**Conclusions**

The ET system plays an important role in cardiovascular physiology and pathophysiology and ET receptor antagonism is now an established therapy for PAH and scleroderma digital ulceration. There remains promise for this therapeutic approach in a number of clinical areas including treatment-resistant hypertension and in the setting of antiangiogenic therapy for cancer. Further investigation into the potential pleiotropic clinical benefits of ET receptor blockade in AKI, CKD and ESRD (especially those patients receiving maintenance haemodialysis) also now merit consideration. More broadly, an important role for the ET system has been demonstrated in the progression of a number of cancers as well as in chronic pain syndromes6. Most of the studies examining the role of ET-1 in specific types of pain are pre-clinical and cover a range of diseases including arthritis, diabetes and sickle cell disease, all of which are associated with a long-term cardiovascular risk. In sickle cell disease, recent data suggest an important role for the ET system in its associated nephropathy and clinical trials in this area are planned121.

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**Author contributions**

N.D. researched data for the article. Both authors discussed the content and wrote the manuscript. D.J.W. reviewed and edited the manuscript before submission.

**Fig. 1** | **Pathways of endothelin 1 (ET-1) synthesis and sites of action.**

The left panel shows promoters of ET-1 synthesis. The right panel shows the pathway for the generation of ET-1. The bottom figure shows sites of ET-1 action. ANP: atrial natriuretic peptide, BNP: brain natriuretic peptide, ETA: endothelin-A receptor, ETB: endothelin-B receptor, IL-1: interleukin-1, LDL: low-density lipoprotein, TGF-β: transforming growth factor β,

**Fig. 2** | **ETA and ETB mRNA expression in different organs.** Relative expression of mRNA encoding ETA (*Ednra*; part **a**) or ETB (*Ednrb*; part **b**) receptors in 41 adult tissues. Reproduced from REF.6. Editorial Team: could this figure be re-drawn to a newer, better looking figure?

**Fig. 3** | **Effects of endothelin 1 (ET-1) in the kidney. a** | **Role of ET-1 in the regulation of glomerular haemodynamics.** Dual ETA/B receptor antagonism is required to fully abolish the vasoconstricting effects of ET-1 on the afferent arteriole suggesting that both ETA and ETB receptors are active here; at the efferent arteriole the effects of ET-1 are blocked by ETA receptor antagonism alone, and enhanced by ETB receptor blockade, suggesting that ET-1 can modulate efferent arteriolar tone via the ETA receptor and that the balance of ETB receptor effects here is to produce vasodilation. By this action on efferent and afferent arterioles, ET has the ability to regulate glomerular capillary pressure and so glomerular filtration. Additionally, ET-1 has been shown to reduce filtration coefficient by mesangial cell contraction. **b** | **Role of ET-1 in podocyte dedifferentiation and proteinuria.** Proteinuria is associated with damage to the podocyte, the highly specialised glomerular epithelial cell that maintains the integrity of the glomerular filtration barrier. Podocytes possess a contractile structure that responds to vasoactive hormones to control glomerular capillary surface area and in turn ultrafiltration coefficient. Podocytes undergo phenotypic changes as a result of exposure to large amounts of protein that resemble dedifferentiation. In parallel, there is increased ET-1 production by the podocyte, which is, at least partly, dependent on the cytoskeletal rearrangements brought about by excess protein exposure. ET-1 can itself bring about similar podocyte cytoskeletal changes as protein loading. Thus, podocyte-derived ET-1, acting in an autocrine and paracrine manner, may promote further podocyte ultrastructural degeneration and hence its own production, with both of these contributing to a further breakdown in the glomerular filtration barrier. Podocyte-derived ET-1 may act on other glomerular cells increasing the tone of the glomerular capillary, enhancing vascular permeability, and stimulating mesangial cell contraction by virtue of its vasoconstrictor effects, all effects leading to glomerular hypertension and a further decline in functional renal mass. **c** | **Role of ET-1 in interstitial fibrosis.** Upregulation of the ET system stimulates tubular protein reabsorption. Also, exposure of proximal tubular cells to a protein load leads to their production of ET-1. As the majority of ET-1 is secreted abluminally one can infer that *in vivo* there is a build-up of ET-1 in the renal interstitium in proteinuric nephropathies. Here, ET-1 may bind to interstitial fibroblasts and promote their proliferation and generation of extracellular matrix, which in turn is capable of further inducing ET-1 synthesis. Furthermore, ET-1 is chemotactic for blood monocytes and leads them to secrete proinflammatory cytokines and growth factors, events that would contribute to interstitial remodelling and scarring. **d** | **Role of collecting duct cell ETB receptor in natriuresis and diuresis.** ET-1 is produced by inner medullary collecting duct cells where it inhibits the AVP stimulated retention of water; extracellular sodium concentrations may regulate collecting duct ET-1 production. The natriuretic role of the tubular ETB receptor is linked to NO generation. A potent inhibitory action of NO on tubular sodium reabsorption is well described. ET-1, acting via ETB and NO, can inhibit chloride transport in the medullary thick ascending limb of Henlé, thus promoting natriuresis. AVP: vasopressin, endothelin-A receptor, ETB: endothelin-B receptor, ECM: extracellular matrix, H20: water, Na+: sodium, NO: nitric oxide, IL-1: interleukin-1, IL-6: interleukin-6, TGFβ: transforming growth factor β, TNFα: tumour necrosis factor α.

**Table 1** | **Targets for contraction and relaxation of vascular smooth muscle cells by endothelin-1 (ET-1) and the receptors involved**

|  |  |  |
| --- | --- | --- |
| **Target** | **Action** | **Receptor** |
| ***Heart*** | | |
| Coronary vasculature | Vasoconstriction | ETA (ETB) |
| Vasodilatation | ETB |
| Cardiomyocyte | Inotropy | ETA (ETB) |
| ***Lung*** | | |
| Pulmonary artery | Vasoconstriction | ETA / ETB |
| Vasodilatation | ETB |
| ***Kidney*** | | |
| Renal artery | Vasoconstriction | ETA |
| Vasodilatation | ETB |
| Afferent arteriole | Vasoconstriction | ETA / ETB |
| Efferent arteriole | Vasoconstriction | ETA |
| Vasodilatation | ETB |
| Cortical vasculature | Vasoconstriction | ETA |
| Medullary vasculature | Vasodilatation | ETB |
| Mesangial cell | Contraction | ETA |
| ***Brain*** | | |
| Cerebral vasculature | Vasoconstriction | ETA |
| Vasodilatation | ETB |

**Table 2** | **Other key actions of endothelin-1 (ET-1) and the receptors involved**

|  |  |  |
| --- | --- | --- |
| **Target** | **Action** | **Receptor** |
| ***Cardiovascular*** | | |
| Cardiomyocyte | Hypertrophy | ETA |
| Vascular smooth muscle cells | Growth/proliferation | ETA |
| Pulmonary artery | Proliferation | ETA / ETB |
| Endothelial cells | ET-1 clearance | ETB |
| ***Kidney*** | | |
| Collecting ducts | Natriuresis | ETB |
| ***Adrenal*** | | |
| Zona glomerulosa | Aldosterone secretion | ETA / ETB |
| ***Inflammatory cells*** | | |
| Macrophages | Inflammation/atherosclerosis | ETA |
| Fibroblasts | Fibrosis | ETA |

**Table 3** | **Characteristics of endothelin receptor antagonists, either licensed or currently under active investigation**

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Drug** | **Indication** | **Dose (mg)** | **Structure** | **Tmax (h)** | **T ½ (h)** | **Clearance** | **ETA selectivity** |
| Bosentan (Tracleer®) | Licensed (PAH, scleroderma and digital ulcers) | 62.5–125 | 1 | 4.5 | 5 | Liver | 20:1 |
| Macitentan (Opsumit®) | Licensed (PAH) | 10 | 2 | 4-12 | 16 | Liver and renal | 800:1 |
| Ambrisentan (Letairis®; Volubris®) | Licensed (PAH) | 5–10 | 3 | 2 | 15 | Liver | 500:1 |
| Aprocitentan (ACT-132577) | Phase III (TRH) | — | 4 | 30 | 48 | Liver | 60:1 |
| Clazosentan | Phase III (SAH) | — | 5 | ? | ? | Liver | 1,000:1 |
| Zibotentan | Phase II (scleroderma) | — | 6 | 2 | 9–25 | Renal | >10,000:1 |
| Sparsentan | Phase II (FSGS) | — | 7 | 3 | 13–17 | Liver | 1,000:1 |

FSGS, focal and segmental glomerulosclerosis; PAH, pulmonary arterial hypertension; SAH, subarachnoid haemorrhage; TRH, treatment-resistant hypertension.

**See PowerPoint slide for ERA structures**