ELSEVIER

Endothelin-1 traps as a potential therapeutic tool: from diabetes to beyond?

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There is substantial research on the vasoactive peptide endothelin (ET)-1 in physiology, as well as in pathology. In fact, pathologically elevated levels of ET-1 have been found in several disease states, such as various cardiovascular diseases, different cancers, some neurodegenerative disorders, as well as in diabetes. Here, we describe and discuss ET-1, its importance in different diseases, and the potential therapeutic effects of ET-traps in the treatment of these diseases. Previous *in vitro* and *in vivo* research (in the diabetes disease space) demonstrated that ET-traps potently and significantly prevent the induction of different markers of diabetes-related pathology. This included induction of extracellular matrix (ECM) proteins (collagen 4α1 and fibronectin), which are pathologically elevated in diabetes. The ET-traps prevented induction of these and brought a significant return to non-diabetic levels. We also discuss the merits of using ET-traps over the currently used endothelin receptor antagonists (ERAs) and previously used therapeutic antibodies.

Introduction

The vascular endothelium is responsible for maintaining local vascular tone by releasing different vasoactive substances. This includes relaxing factors, such as nitric oxide, prostacyclin, and endothelium-derived hyperpolarizing factors, as well as the potent vasoconstrictor peptide ET-1 [1].

What is endothelin-1

ET-1 is a 21-amino acid peptide that is synthesized and secreted by a range of cells [2]. Previous research showed that ET-1 is able to induce oxidative stress, endoplasmic reticulum (ER) stress, as well as inflammation. ET-1 induces its actions when it binds the endothelin A or B receptor. These are both G-protein-coupled receptors. Serum or tissue levels of ET-1 are pathologically elevated in a host of different diseases, including cardiovascular diseases, neurodegenerative disorders, cancers, as well as pregnancy disorders in addition to diabetes [3]. Increased levels of ET-1 in these diseases trigger the induction of different pathological processes, including inflammation, oxidative stress, and ER stress. As such, different ERAs are in use to prevent the induction of these processes, which lead to disease pathology [4].

Endothelin-1 in diabetes

Type 1 diabetes mellitus (T1DM) is a metabolic disorder that develops over several years and is characterized by a lack of insulin production because of the specific destruction of pancreatic β cells [5]. In addition to hyperglycemia, disruptions in angiogenesis can cause, or contribute to, many of the clinical manifestations of diabetes. As previously described, various clinical studies show a significant increase in ET-1 levels in diabetes compared with normal, control subjects [6,7]. Patients with diabetes are associated with endothelial dysfunction, which leads to increased production of the potent vasoconstrictor and proinflammatory peptide ET-1 [8]. Diabetes is associated with increased expression of ECM proteins, such as fibronectin and collagen 4α1, in key tissues and organs, such as heart and kidneys [9].

What are ET-traps?

ET-traps are molecular constructs, fused to the Fc portion of immunoglobulin (Ig)-G1, that potently bind and sequester ET-1 with (initially) a binding affinity in the nanomolar range [10]. After modifications, this

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was increased to picomolar binding affinity to ET-1 [11]. Hence, they offer a useful tool for binding and sequestering (pathologically) elevated ET-1 levels. A binding affinity in the picomolar range means that they more strongly bind ET-1 than other agents currently in use [12,13].

Therapeutic antibodies that have an isoelectric point close to the pH of human blood have been found to be most effective in penetrating tissue [14]. The predicted isoelectric point of ET-traps is 7.67, which is similar to the pH of human blood. This enables them to have an efficacious effect on heart and kidney tissues in an (*in vivo*) study on diabetes [9].

ET-traps in diabetes

As mentioned earlier, diabetes is associated with increased plasma levels of ET-1. This aggravates the expression of the ECM proteins. The increased levels of ET-1 also have a deleterious effect in diabetes on the heart, termed 'diabetic cardiomyopathy'.

ET-traps have a therapeutic effect in diabetes. They result in a significant reduction in collagen 4α1 expression in the heart and kidney back to control, non-diabetic levels at both the mRNA and protein level [9]. The expression of fibronectin mRNA is also returned to control levels with ET-traps administered via subcutaneous injection. The beneficial use of ET-traps in diabetes was also shown by histological data, echocardiograph studies, and a measure of urinary creatinine and albumin levels. In all analyses, the ET-traps returned the different measures to control, non-diabetic levels [9]. Furthermore, ET-traps are not immunogenic and are not associated with any adverse effects, which are important factors when developing a therapeutic.

In vivo work in diabetes also showed that ET-traps had a beneficial effect on heart and kidney tissues (as seen in the immunohistochemistry analyses of these tissues), echocardiography measures, as well as measures of urinary markers of kidney function [9]. Therefore, ET-traps have a broad therapeutic effect and would likely be useful for a host of other diseases associated with pathologically increased ET-1.

ET-1 is implicated in different diseases

Increased ET-1 in cardiovascular diseases

Pathologically elevated ET-1 levels are found in different cardiovascular diseases, including hypertension, heart failure, and atherosclerosis [15].

Quynh *et al*. discussed the role of inflammation and oxidative stress in vascular dysfunction in hypertension [16]. The authors also discussed how endothelial dysfunction results. Endothelial dysfunction is broadly defined as an imbalance between vasodilating and vasoconstricting substances produced by the endothelium [17]. As previously discussed, endothelial dysfunction is a source of ET-1, which could be a source of the increased levels of ET-1 prevalent in hypertension. Elevated ET-1 levels would constrict arteries and contribute to an increased blood pressure, leading to damage to different organs in the body [17].

Over time, hypertension can eventually lead to heart failure (www.heart.org/en/health-topics/high-blood-pressure/health-threats-from-high-blood-pressure/how-high-blood-pressure-can-lead-to-heart-failure). Heart failure is associated with aberrant systolic function and diastolic dysfunction [18]. Heart failure results from structural and functional defects in the myocardium, which results in impairment of ventricular filling or the ejection of blood, which results in reduced left ventricular myocardial function. Heart failure is also associated with diastolic dysfunction [21]. A previous *in vivo* study showed that ET-traps treatment significantly returned to non-pathological levels different echocardiography markers, including measures of left ventricular systolic function in the ejection fraction (EF) and fractional shortening (FS) or diastolic dysfunction (E/A and E'/A' ratios) [9].

The efficacy of the use of an ERA shows that ET-1 is responsible for the pathology in hypertension. Treatment of other cardiovascular diseases, including heart failure and atherosclerosis, also derive benefit from the use of ERAs [8,19,20] (www.cvpharmacology.com/vasodilator/ETblockers), again highlighting ET-1 as a significant underlying pathological factor in cardiovascular diseases. Later, we discuss why ET-traps offer a superior option to other therapies targeting the endothelin system, including treatments that are already in use.

Increased ET-1 in CKD

Chronic kidney disease (CKD) is a condition characterized by the gradual loss of kidney function over time (www.kidney.org/atoz/content/about-chronic-kidney-disease). CKD affects 10% of the population worldwide (www.worldkidneyday.org/facts/chronic-kidney-disease/). Approximately half of individuals living with CKD also have diabetes or cardiovascular disease. Albuminuria is increased in CKD and is a measure of kidney damage. Therefore, as per recommendation, albuminuria levels are tested in adults with CKD or those at risk for CKD. This is considered the gold standard. In a diabetes study by Jain *et al*., ET-traps significantly reduced the deposition of ECM proteins in the kidneys of diabetic mice, indicating improved kidney function [9]. Furthermore, there was a significant improvement in urinary albumin and creatinine levels excreted by diabetic mice treated with ET-traps [9]. In fact, all the improvements seen in kidney function were statistically significant and showed a return to normal, control levels. Therefore, it is likely that ET-traps could be used as a treatment for CKD. Diabetic nephropathy is the leading cause of end-stage CKD or ESRD [21], which is a major health issue: one study described that, in the USA alone, the costs for treating patients with CKD who are older than 65 is more than US$45 billion, illustrating the economic burden associated with CKD. Patients with ESRD require lifelong dialysis and the only possible treatment is kidney transplant [22]. The results of the study by Jain *et al*. indicate that ET-traps would help prevent progression of CKD and, thus, could be a potential therapy for disease. Urinary albumin or albumin/creatinine ratio (ACR) is used as a screening for individuals with chronic conditions, such as diabetes and hypertension to test for any kidney disease development. Jain *et al*. reported increased levels of creatinine and albumin in the urine of diabetic mice, which were significantly reduced in diabetic mice treated with ET-traps. A recent study reported the use of an ERA for the treatment of diabetic nephropathy [23]. The different clinical trials with ERAs in diabetic nephropathy highlight the importance of targeting the endothelin system in this disease.

Increased ET-1 in neurodegenerative disorders

Previous studies have reported significantly elevated levels of ET-1 in neurodegenerative diseases, including Alzheimer's disease (AD), Parkinson's disease (PD), and multiple sclerosis (MS) [3]. D'haeseleer *et al*. showed that increased levels of ET-1 in MS

resulted in reduced cerebral blood flow (CBF) [24]; given that ET-1 is a potent vasoconstrictor [25], it is unsurprising that it can compromise CBF. D'haeseleer *et al*. reported that using bosentan (an ET antagonist) reversed certain markers causing disease pathology. For example, CBF was significantly lower in patients with MS than in control subjects and increased to control values after bosentan administration. Therefore, the reduced CBF in MS appears to be mediated by ET-1. Therefore, ET-traps, which potently and significantly bind and reduce the levels of circulating ET-1, could become a potential therapy for MS.

In addition to the reduction in CBF caused by circulating ET-1, the increase in ET-1 levels in the brain itself is caused by the release of ET-1 from astrocytes in brain lesions. It remains to be tested whether ET-traps can cross the blood–brain barrier (BBB). Previous work showed that not only the size, but the level of how hydrophobic a molecule is both determine whether a molecule would be able to cross the blood–brain barrier [26]. Given that the ET traps are both small in size (37.1 kDa) and are hydrophobic in nature (37.1%), it is likely that they would be able to cross the BBB. Furthermore, as has been previously reported [9], the ET-traps potently and significantly sequester increased ET-1 levels in the blood. This in turn would reduce the levels of the increased reactive white blood cells that freely cross the cerebral spinal fluid and BBB. The white blood cell count is increased by inflammation [27] and ET-1 is a potent inducer of inflammation [28]. This increase in inflammation is characterized by increases in the levels of reactive white blood cells [28] and systemic inflammation, which affects different organs in the body [29]. The various neurodegenerative disorders mentioned earlier are all associated with systemic inflammation. Thus, a tool such as ET-traps could have an efficacious effect by reducing pathologically elevated ET-1 levels.

In addition to MS, there is much evidence suggesting an important role for systemic inflammation in the pathogenesis of Alzheimer's disease (AD). AD is a common cause of dementia, accounting for ~60–90% of all cases [30]. Different disease-modifying therapies for AD, such as immunotherapy against amyloid beta (Aβ), are now under investigation and have been tested in clinical trials, although with little success so far. Therefore, there is an urgent need to identify new therapeutics. Previous AD research using disease models and clinical studies has demonstrated a significant contribution of inflammation in general to pathological features and symptoms of AD.

Systemic inflammation is also implicated in Parkinson's diseases (PD) [31,32]. Ferrari and Tarelli reported that peripheral (systemic) inflammation triggers exacerbation of damage in the brain in several neurodegenerative diseases [32]. Chiang *et al*. revealed that damage to the white matter of patients with PD is associated with systemic inflammation [31].

Given the increased levels of ET-1 in different neurodegenerative disorders and that this increased ET-1 is a cause of pathology in these diseases, ET-traps, which potently and significantly reduce the circulating levels of ET-1, would have a therapeutic effect in ameliorating disease pathology.

Increased ET-1 disorders of the eye

ET-1 occurs in the human corneal epithelium, and its levels are increased in eye disorders [33–35]. Previous research showed the presence of nitric oxide, a relaxing factor, and ET-1, a vasoconstrictor, in the human ophthalmic artery [36]. These (endothelium-derived) vasoactive substances have an important role in the regulation of the human ophthalmic circulation. Therefore, a tool such as ET-traps that significantly reduces blood ET-1 levels could act as a therapeutic for eye disorders. In support of this, it was previously suggested that an increase of ET-1 in circulating blood leads to the local constriction of retinal veins [37].

Increased ET-1 in human cancers

Increased (plasma) levels of ET-1 are found in patients with various solid tumours, including hepatocellular, gastric, and prostate cancers [38–40]. Previous work showed that ET-1 modulates mitogenesis, apoptosis, angiogenesis, tumor invasion, and development of metastases [41]. Various ERAs have been evaluated as therapeutic agents in cancer. ET-1 receptor blockade with ERAs results in antitumor activity by concomitant growth inhibition and apoptosis induction. Small-molecule antagonists for targeting endothelin receptors have been evaluated in several recent clinical trials. However, results to date have been disappointing and, thus, it is crucial to decipher why promising preclinical data have not yet been translated to the clinic [42]. It could be that complete antagonism of the endothelin receptors is associated with adverse effects, given that the functions of ET-1 are crucial for normal physiology. In addition, each of the two endothelin receptors is responsible for different cellular mechanisms and, therefore, using an inhibitor drug to only one of these receptors is not efficacious. Hence, dual endothelin receptor antagonists, such as macitentan, are currently in use because they exhibit higher efficacy [43]. Similarly, the ET-traps would also have an effect on both receptors, because they sequester the pathologically elevated ET-1 levels found in the different cancers, thereby targeting the actions of ET-1 through either receptor. In addition, because the ET-traps would not disrupt the normal, physiological functions of ET-1, they would not be associated with adverse effects. Neither adverse effects nor toxic effects have been reported so far in *in vivo* work with ET-traps in diabetes [9].

Increased ET-1 in pregnancy disorders

Pre-eclampsia is a pregnancy disorder characterized by hypertension [44], in which levels of ET-1 are pathologically elevated [45]. The treatment of pre-eclampsia is problematic and mainly aims to minimize the symptomatic complications of the disease [44].

Given that research has shown that ET-1 is a primary pathological factor in pre-eclampsia [4,45], ET-traps could be used as a novel therapy for pre-eclampsia. Previous research has shown that different ERAs have an efficacious effect on blocking different pathological processes that are implicated in pre-eclampsia [45–47]. For example, BQ788, which is an ETB receptor antagonist, has a positive effect in reducing endoplasmic reticulum (ER) stress [48]. These studies highlight the importance of targeting the ET system.

Given that testing new drugs is an issue in pregnancy, especially because it is uncertain how much of that new drug would reach the fetus, it would be helpful to test this first *ex vivo* using, for example, the placental perfusion model [49] that is an appropriate model for pre-eclampsia [50]. Therefore, the efficacy of the ET-traps could be tested in the pre-eclampsia disease space by checking their effect on different markers of pathology in both the tissue and the

(maternal and fetal) perfusates, including ET-1 levels. This system could also be used to test how many of the ET-traps cross the placenta and reach the fetus by adding a dose to the maternal circuit (perfusate) and then measuring how many of the ET-traps cross the placenta to reach the fetus (measured in the fetal perfusate) over a time course experiment.

Although a human placenta has been used as an example to illustrate damage that might result from disrupting the endothelin system, this would also be true for other disease states, especially with the usage of ET-1 antagonists over time (Fig. 1). Traditional ERAs (inhibitor drugs) might positively impact the organ of concern (e.g. the placenta), but given that ET-1 is necessary for normal, physiological functions, there might be damage to other organ systems.

Use of ERAs is restricted during pregnancy, because the endothelin system is required for normal fetal development; Kurihara *et al*. demonstrated that fetal mice that were deficient in ET-1 or administered bosentan went on to develop hypertension and severe craniofacial abnormalities [51]. Deficiency in ET-1 has also been implicated in premature closure of the ductus arteriosus in preclinical models [52]. The safety of macitentan (along with that of other ERAs) has not been ascertained in the pediatric population and, thus, it is not approved for use in these patients [12,53]. However, T1DM can occur in children and is often referred to as 'juvenile' diabetes [54]. In this case, the use of ET-traps would overcome issues related to complete blockage of the endothelin system.

Increased ET-1 in other diseases

Various other diseases are associated with increased ET-1 levels, such as systemic lupus erythematosus, and other autoimmune diseases [55,56]. Thus, it is likely that ET-traps have a therapeutic effect in all these diseases. However, their efficacy needs to be tested and confirmed.

ET-traps versus current endothelin based therapeutics

Current treatment options based around ET-1 include ERAs. These are used in different cardiovascular diseases, as well as cancers [8,43], among others. The successful use of ERAs in these diseases highlights the prominent role of ET-1 in those diseases where it is pathologically elevated. Here, we discuss the advantages of ET-traps over ERAs or therapeutic antibodies to ET-1.

ET-traps versus ERAs

ERAs block one or both of the endothelin-1 receptors (ETA or ETB receptors). By contrast, ET-traps do not completely block the activity of ET-1 because it is crucial for different physiological processes; instead, ET-1 is sequestered as close to normal levels as possible. Another advantage is the less frequent dosing requirements of ET-traps. For example, the dissociation of

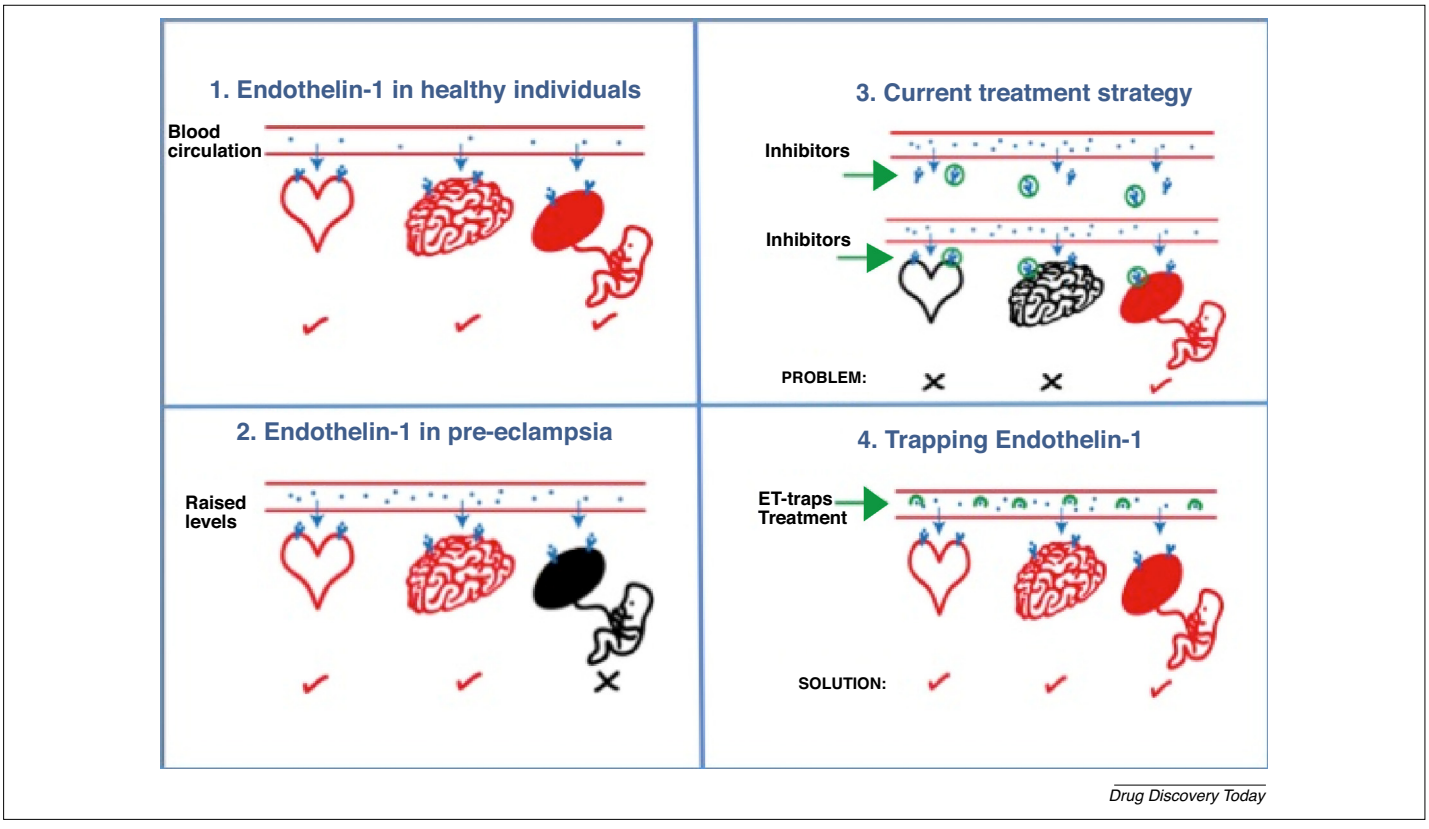

FIGURE 1

ET-traps do not completely block the essential functions of endothelin-1 (ET-1). **(a)** ET-1 levels in a normal individual. **(b)** increased ET-1 levels in disease, in this case affecting the placenta in pre-eclampsia, whereas other organs function as normal. **(c)** Current treatment strategy using inhibitor drugs or ERAs, which might act not only on the target tissue, but also other tissues, given that ET-1 is essential for different physiological functions. **(d)** The use of ET-traps overcomes this issue.

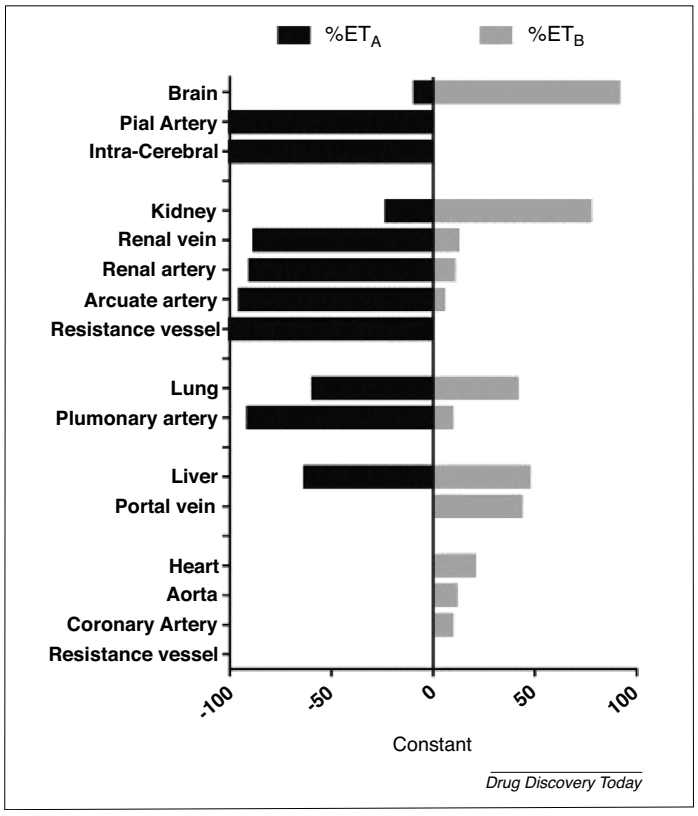

FIGURE 2

Differential expression of the endothelin (ET)-A and ETB receptors. Radioligand labeling showed that varying amounts of the ETA and ETB receptors are expressed in different organs. Reproduced, with permission, from Ref. [57] (https://creativecommons.org/licenses/by/4.0/).

macitentan once bound to the ETA or ETB receptors is 17 min [13]. Although this is many-fold higher than that of other agents, such as bosentan (receptor dissociation of 70 s), macitentan still requires daily dosing. By contrast, the dissociation of ET-traps is approximately 60+ min and they only require administration three times a week to have an efficacious effect [9]. Therefore, with higher binding affinity and slower dissociation compared with ERAs, the dosing of ET-traps for therapeutic use is much lower.

In addition, ET-traps are not associated with any known adverse effects or toxicity [9,11]. By contrast, ERAs are associated with adverse effects ranging from mild to severe. For example, bosentan and sitaxentan can exhibit liver toxicity (https://livertox.nih.gov/Macitentan.htm#other_refs). Finally, a previous study showed differential expression of the ETA and ETB receptors [57] (Fig. 2). For example, the ETA receptor shows higher expression in the brain. Therefore, a tool such as ET-traps would be more beneficial than an ERA specific to either of the two receptors, because it would have an effect upstream, targeting pathologically elevated ET-1 levels in a given condition.

ET-traps versus therapeutic antibodies to ET-1

Fc-fusion proteins (FFPs), which are our ET-traps, have a longer serum half-life compared with therapeutic antibodies to ET-1. Therefore, FFPs are able to persist for longer to bind their targets and exert their therapeutic effects. FFPs can also be modulated more easily, compared with therapeutic antibodies, so that the therapy does not elicit any negative immune reaction. Finally, in terms of an economic comparison, tumor necrosis factor (TNF)-α FFP has ~US$7.6 billion sales per year, almost US$1 billion higher than the most successful therapeutic antibodies [58], illustrating the value of Fc-based therapeutics.

Concluding remarks

Therefore, ET-traps could provide a useful treatment option for a host of different diseases in which pathologically elevated ET-1 is a feature, including cardiovascular diseases, neurodegenerative disorders, and pregnancy disorders, among others not discussed herein. The proof of concept (at the *in vitro* and *in vivo* levels) has been established in the diabetes disease space. Given that various other diseases also have pathologically elevated levels of ET-1, it is likely that the ET-traps could also be used in these disease areas, although such use requires testing in relevant animal models. How the ET-traps might be differentiated for use in these different diseases will be modulated by the strength and frequency of dosing. Building on the work described in this review, future studies will help realize the potential of ET-traps as a therapy for different diseases.

Conflict of interest

A.J. and V.M. are members of Accelerate Cambridge, University of Cambridge, UK.

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