In vivo studies demonstrate that endothelin-1 traps are a potential therapy for type 1 diabetes

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Abstract

Background Type 1 diabetes is a serious, lifelong condition where the body's blood glucose level increases because of the body's inability to make insulin. An important consequence of this is the increased expression of extracellular matrix proteins, such as fibronectin and collagen 4 α 1, in key tissues and organs like the heart and kidneys. Diabetes is also associated with increased plasma levels of the vasoactive peptide endothelin (ET)-1. This further aggravates the expression of the ECM proteins. There are also important consequences of increased glucose and ET-1 levels in diabetes on the heart, termed diabetic cardiomyopathy.

Methods We have previously reported the development of ET-traps, which potently and significantly reduce pathological levels of ET-1. In this study, we tested the in vivo therapeutic potential of ET-traps for type 1 diabetes using the B6 mouse model.

Results Following subcutaneous administration of ET-traps 3 times a week, over a 2 month period, the 500 nM dose of ET-traps gave a significant reduction in collagen 4 α 1 expression in the heart and kidney, returning it back to control, non-diabetic levels at both the mRNA and protein levels. The expression of fibronectin mRNA is also returned to control levels with the 500 nM dose of ET-traps. The efficacy of ET-traps for type 1 diabetes was further evinced by immunohistochemistry data, echocardiography studies (measuring left ventricular systolic function and diastolic dysfunction) and a measure of urine creatinine and albumin levels. In all analyses, the 500 nM dose of ET-traps returns the different measures to control, non-diabetic levels.

Conclusion Data from this study show that in a mouse model ET-traps have a potent and significant therapeutic effect on diabetes disease pathology. Future studies could further evaluate the use of ET-traps as a therapy for diabetes, including taking them through clinical trials.

Keywords DM, Diabetes mellitus · ECM, Extracellular matrix · ET-1, Endothelin-1 · ETtr, Endothelin-1 traps · FFP, Fc-fusion protein

Key points

ET-traps have a very high binding affinity.
ET-traps significantly return different markers of pathology to basal levels (have a therapeutic effect) in an *in vivo* model of diabetes.
ET-traps are non-toxic.

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Abbreviations

ALT	Alanine aminotransferase
AST	Aspartate transaminase
CKD	Chronic kidney disease
DTI	Doppler tissue imaging
ET-1	Endothelin-1
ECM	Extracellular matrix
ESRD	End-stage renal disease
ETtr	Endothelin-1 traps
FN	Fibronectin
STZ	Streptozotocin
T1D	Type 1 diabetes

Introduction

Type 1 diabetes (T1D) is an autoimmune disease with a strong genetic component [1, 2]. T1D can occur at any age but tends

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to develop in childhood [3]. T1D is characterized by destruction of pancreatic β -cells, which results in absolute insulin deficiency [4]. A survey in 2014 stated that an estimated 387 million people have diabetes worldwide [5], of which T1D accounts for between 5% and 10% [6].

Elevated glucose or altered insulin sensitivity have an important effect on cellular components within the heart. In addition, there are also significant changes in the cardiac extracellular matrix (ECM) [7]. A key feature of chronic diabetes is an imbalance in the production and degradation of extracellular matrix (ECM) proteins, such as fibronectin (FN) and collagen 4 α 1. The mechanism of increased ECM synthesis is important for the development of therapeutics, as ECM deposition is a common occurrence in chronic diabetes [8]. Research shows that high glucose-induced fibronectin synthesis, which is mediated via NF- κ B and AP-1, is dependent on endothelin (ET)-1 [9]. The study by Chen et al. (2003) showed that ET receptor blockade, which inhibits ET-1 activity, prevented the high glucose-induced fibronectin synthesis. Therefore, the ET-traps would also have a positive effect in diabetes because they would potentially help reduce the (increased) levels of ET-1, as has been shown by our previous work [10]. ET-1 also increases collagen 4 α 1 accumulation in renal mesangial cells by stimulating a chemokine and cytokine autocrine signaling loop, which involves the macrophage chemoattractant protein-1 (MCP-1) and interleukin-6 (IL-6) [11].

Various studies show that ET-1 levels are significantly increased in diabetes compared to control subjects [12, 13]. Endothelial cell dysfunction is a key pathological element in the development of chronic diabetic complications [14, 15]. Endothelial cell dysfunction involves an imbalance between vasodilating and vasoconstricting substances produced by (or acting on) the endothelium [16]. Endothelial dysfunction leads to increased production and biological activity of the potent vasoconstrictor and pro-inflammatory peptide ET-1 [17].

A previous study found that chronic activation of PKC- δ in cardiac tissues is detrimental to cardiac function and may contribute to diabetic cardiomyopathy. The study by Zhiang He [18] suggested that this was due to the increased gene expression of ET-1 in the myocardium. The role of ET-1 in diabetic cardiomyopathy has been corroborated by further studies using ET antagonists [19]. These previous data show that sequestering pathologically elevated ET-1 has a positive effect on diabetic cardiomyopathy. We therefore, investigated the efficacy of ET-traps in ameliorating diabetic cardiomyopathy.

Further, diabetes mellitus is the most frequent cause of chronic kidney failure in both developed and developing countries [20]. Nephropathy is when the kidneys start to incur damage, which can ultimately lead to kidney failure. Diabetic nephropathy is a clinical syndrome characterized by albuminuria and increased creatinine levels [21, 22]. Previous studies

have reported endothelin antagonism as a therapeutic tool to reduce proteinuria and delay kidney injury progression in diabetic nephropathy [23]. Hence, we also measured albumin and creatinine levels in subjects treated with different concentrations of ET-traps (which would only sequester pathologically high ET-1, without completely blocking its action).

The ET-traps are an Fc-fused molecular construct that potentially bind and sequester ET-1 (as shown by our previous work [10]). The aim of the current study was to evaluate the efficacy of ET-traps in type-I diabetes. For this, we looked at FN and collagen 4 α 1 mRNA and protein expression levels in the heart and kidneys, performed echocardiography analyses, examined the urine creatinine and albumin levels and performed immunohistochemical analyses of the heart and kidney tissues in B6 mice treated with different doses of the ET-traps.

Materials and methods

Please note, the in vivo testing work was contracted out to a University in Canada (to a laboratory with expertise in diabetes research) in order to overcome any conflicts of interest or bias in performing the experiments and analyzing the data. The in vivo testing work was performed by Professor Chakrabarti's laboratory at the Western University, Canada.

Animal studies

All animal experiments were performed according to the *Guide For the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH publication no. 85–23, revised in 1996). The Western University council on animal care committee reviewed and approved all experimental protocols. Male B6 mice (20–25 g) were divided into 1:control, 2:diabetes, 3:diabetes plus 250 nM ET-traps and 4:diabetes plus 500 nM ET-traps groups. Each group contained six animals, i.e. $n = 6$. Diabetes was induced by upto 5 intraperitoneal injections of Streptozotocin (STZ) (50 mg/kg in citrate buffer, pH 4.5, controls received the same volume of buffer) on consecutive days as previously described [24]. Hyperglycemia was confirmed by measuring blood glucose levels (at least twice) 3 days after the last STZ injection. Following confirmation of diabetes, treatment groups received 250 nM or 500 nM ET-traps via a subcutaneous injection on alternate days. The mice were maintained in a hyperglycemic state and followed up for two months (without exogenous insulin) with body weight and glucose. At the end of the study period, echocardiography was performed and 24 h urine were collected in all mice. The mice were then sacrificed and cardiac tissues, renal tissues and blood were collected. The tissues were embedded in paraffin. For histologic studies, 5 μ m sections with hemotoxylin/eosin, PAS-D and trichrome

stain were used, using a standard histological approach as previously described [25].

Echocardiography

As previously described by Feng et al. (2017), lightly anesthetized (1.5% inhaled isoflurane) mice were subjected to echocardiography on a warm handling platform [26]. A 40 MHz linear array transducer (MS-550D) and Vevo 2100 preclinical ultrasound system (VisualSonics, Toronto, Canada) with nominal in-plane spatial resolution of 40 μm (axial) $\times$ 80 μm (lateral) was used. M-mode and 2-D parasternal short-axis scans (133 frames/s) at the level of the papillary muscles were obtained to assess changes in left-ventricular (LV) end-systolic inner diameter (LVIDs) and LV end-diastolic inner diameter (LVIDd). LV volumes at end-diastole (LVEDV) and end-systole (LVESV) were calculated as: $\text{LVEDV} = [7/(2.4 + \text{LVIDd})] \times [\text{LVIDd}]^3$ and $\text{LVESV} = [7/(2.4 + \text{LVIDs})] \times [\text{LVIDs}]^3$. LV fractional shortening (FS) and ejection fraction (EF) were used as indexes of cardiac contractile function and were calculated from the inner diameters as: $\text{FS} [\%] = (\text{LVIDd} - \text{LVIDs}) / \text{LVIDd} \times 100$, and EF as $[\%] = (\text{LVEDV} - \text{LVESV}) / \text{LVEDV} \times 100$, respectively. Color flow-guided, pulsed-wave (PW) Doppler recordings of the maximal early (E) and late (A) diastolic transmitral flow velocities and Doppler tissue imaging (DTI) recordings of peak E' velocity and peak A' velocity were carried out. Mitral inflow patterns (E/A ratio) and mitral annulus velocities (E'/A' ratio) were used to assess diastolic dysfunction [26].

RNA isolation and cDNA synthesis

Trizol reagent (Invitrogen, Burlington, Canada) was used to isolate RNA as previously described [27]. Chloroform was used to extract RNA, which was followed by centrifugation to separate the sample into aqueous and organic phases. RNA was recovered from the aqueous phase by isopropyl alcohol precipitation and suspended in diethylpyrocarbonate-treated water. Total RNA (5 μg) was used for cDNA synthesis with high capacity cDNA reverse transcription kit (Applied Biosystems, Foster City, USA). The resulting cDNA products were stored at -20°C .

Real-time RT-PCR

Real-time RT-PCR was performed using the LightCycler (Roche Diagnostics Canada, Laval, Canada), as previously described [28]. For a final reaction volume of 20 μL , the following reagents were added: 10 μL SYBR Advantage qPCR Premix (Clontech, Mountain View, USA), 1 μL each of forward and reverse 10 $\mu\text{mol/L}$ primers (Collagen 4 α 1, Fibronectin and β -actin, 7 μL H_2O , and 1 μL cDNA template. The standard curve method was used to quantify the mRNA

levels. Standard curves were constructed by using serially diluted standard template. The data were normalized to β -actin mRNA to account for differences in reverse transcription efficiencies and the amount of template in the reaction mixtures.

Primers used:

Mouse fibronectin primer: [forward: 5-CGGTAGGA CTTCTATTCCT-3, reverse: 5-GATACATGACCCCT TCATTG-3]

And

Mouse collagen 4 α 1 primer: [forward: 5-CCCTGGCT TCAAAGGTGATA-3, reverse: 5- GGGGATCC TGGTATTCCACT-3]

Enzyme-linked immunosorbent assay

Heart and Kidney tissues were homogenized with RIPA lysis buffer and tissue proteins were extracted. Protein concentrations were measured using the BCA protein assay kit (Fisher Scientific, Burlington, ON, Canada). Enzyme-linked immunosorbent assays were performed using commercially available enzyme-linked immunosorbent assay kits for Collagen IV alpha1 (Col 4 α 1, Cloud-Clone Corp. TX, USA) and Fibronectin (Boster Biological Technology, CA, USA) according to the manufacturer's instructions.

Urine albumin and creatinine assay

24 h urine was collected from the animals by placing them in metabolic cages before euthanasia with care so that faeces did not contaminate the urine samples. The measurement of urine albumin and creatinine was performed using an ELISA (Albuwell M; Exocell and The Creatinine Companion, Philadelphia, PA). All procedures were performed according to the manufacturer's instructions, and the data were used to calculate the urine albumin/creatinine ratio as previously described [24].

Immunohistochemistry

For immunohistochemical staining, the de-paraffinized kidney and heart sections were antigen-retrieved in citrate buffer and incubated with primary antibodies overnight at 4°C . The primary antibodies (Abcam) used were rabbit anti-mouse FN and Col 4 α 1 (1:100). The slides were incubated in ImmPRESS (peroxidase) anti-rabbit IgG Reagent kit (Vector Laboratory) for 30 min, mounted, and viewed under a microscope. The methodology was as previously described [25].

Statistical analysis

Statistical significance was determined by Student's *t* test or analysis of variance (Fisher's Protected Least Significant Difference test).

Data are expressed as means $\pm$ SE. A *p* value of ≤ 0.05 was considered to be significant, and results are expressed as the average of *n* = 6 animals/group. The statistical analysis was done using GraphPad Prism 5 (GraphPad) software.

Results

Our preliminary experiments confirmed that the ET-traps have a binding affinity of pico molar affinity (Fig. S1), which was analogous to our in vitro study. We then proceeded to test in vivo toxicology and bioavailability of the ET-traps. These studies showed that the ET-traps are non-toxic to the animals

(B6 mouse model). Histological analyses showed no cellular damage in the heart, lung, kidney, liver, cortex and hippocampus with the higher 500 nM dose of ET-traps (Fig. S2a and S2b). Alanine aminotransferase (ALT) and aspartate transaminase (AST) tests showed no significant decline in liver function (Fig. S2c).

Bioavailability analyses indicated that following subcutaneous injection 3 times per week, there was a significant reduction in plasma ET-1 levels in ET-traps treated mice compared to untreated controls and a significant increase in plasma ET-traps (Fig. S3a and S3b).

ET-traps potently and significantly reduce the expression of col 4 α 1 in the heart of diabetic mice

We tested the efficacy of the ET-traps on ECM protein expression in an in vivo model of diabetes – the B6 mouse model. Our results show that there is a significant increase in heart

Fig. 1 ET-traps potently and significantly reduce the expression of Col 4 α 1 in the heart of diabetic mice back to control, non-diabetic levels. **a** Protein expression analyses (ELISA) of Col 4 α 1 in B6 mouse subjects treated with or without ET-traps. Col 4 α 1 protein levels were significantly increased in the heart of diabetic mice at the end of the 8-week study period. This increase was absent in subjects treated with 500 nM ET-traps. **b** Real time PCR analysis of Col 4 α 1 in the heart. Col 4 α 1 mRNA levels were significantly increased in the heart of diabetic mice at the end of the 8-week study period. This increase was absent in subjects treated with

500 nM ET-traps. The data were normalized to β -actin mRNA to account for differences in reverse transcription efficiencies and the amount of template in the reaction mixtures. Data are presented as means $\pm$ SD. Statistical significance of differences between groups was tested with Student's *t* test. A *p* value of 0.05 or less was considered to be significant. * *p* ≤ 0.05 compared to non-diabetic controls, ** *p* ≤ 0.05 compared to diabetic subjects, *n* = 6, C = control, D = diabetic, 250 = 250 nM ET-traps, 500 = 500 nM ET-traps

Col 4 α 1 in diabetic mice compared to controls (Fig. 1a, b). This was found at both the mRNA and protein levels. There was a significant return of heart Col 4 α 1 to control (non-diabetic) levels with a 500 nM dose of ET-traps, at both the mRNA and protein levels (Fig. 1a, b).

ET-traps potently and significantly reduce the expression of col 4 α 1 in the kidney of diabetic mice

Analogous to the heart, there is a significant increase in kidney Col 4 α 1 in diabetic mice compared to non-diabetic controls (Fig. 2a, b). This was found at both the mRNA and protein levels. There was a significant return of kidney Col 4 α 1 to control (non-diabetic) levels with a 500 nM dose of ET-traps (administered 3 times per week over the 8 week test period), at both the mRNA and protein levels (Fig. 2a, b).

ET-traps potently and significantly reduce the mRNA expression of fibronectin in the kidney of diabetic mice

There is a significant increase in kidney FN mRNA in diabetic mice compared to non-diabetic controls (Fig. 3). There was a significant reduction of kidney FN mRNA (close to control, non-diabetic) levels with a 500 nM dose of ET-traps, administered 3 times per week over the 8-week test period (Fig. 3).

There is a significant increase in urinary markers of pathology that is ameliorated in subjects treated with ET-traps

Measure of urine creatinine and albumin are standard markers of pathology in chronic diabetes. Indeed, in this study, we found a statistically significant increase in urinary albumin,

Fig. 2 ET-traps potently and significantly reduce the expression of Col 4 α 1 in the kidney of diabetic mice back to control, non-diabetic levels. **a** Protein expression analyses (ELISA) of Col 4 α 1 in B6 mouse subjects treated with or without ET-traps. Col 4 α 1 protein levels were significantly increased in the kidneys of diabetic mice at the end of the 8-week study period. This increase was absent in subjects treated with 500 nM ET-traps. **b** Real time PCR analysis of Col 4 α 1 in the kidney. Col 4 α 1 mRNA levels were significantly increased in the kidneys of diabetic mice at the end of the 8-week study period. This increase was absent in subjects

treated with 500 nM ET-traps. The data were normalized to β -actin mRNA to account for differences in reverse transcription efficiencies and the amount of template in the reaction mixtures. Data are presented as means $\pm$ SD. Statistical significance of differences between groups was tested with Student's *t* test. A *p* value of 0.05 or less was considered to be significant. * *p* $\leq$ 0.05 compared to non-diabetic controls, ** *p* $\leq$ 0.05 compared to diabetic subjects, *n* = 6, C = control, D = diabetic, 250 = 250 nM ET-traps, 500 = 500 nM ET-traps

Fig. 3 ET-traps potently and significantly reduce the mRNA expression of fibronectin in the kidney of diabetic mice back, close to control, non-diabetic levels. Real time PCR analysis of fibronectin in the kidney. Fibronectin mRNA levels were significantly increased in the kidneys of diabetic mice at the end of the 8-week study period. This increase was significantly lower in subjects treated with 500 nM ET-traps. The data were normalized to β -actin mRNA to account for differences in reverse

transcription efficiencies and the amount of template in the reaction mixtures. Data are presented as means $\pm$ SD. Statistical significance of differences between groups was tested with Student's *t* test. A *p* value of 0.05 or less was considered to be significant. * $p \leq 0.05$ compared to non-diabetic controls, ** $p \leq 0.05$ compared to diabetic subjects, $n = 6$, C = control, D = diabetic, 250 = 250 nM ET-traps, 500 = 500 nM ET-traps

creatinine and in the albumin to creatinine ratio in diabetic compared to control subjects (Fig. 4a–c). In all cases, there was a statistically significant reduction in all 3 markers of pathology with the 500 nM dose of ET-traps.

ET-traps have a positive effect on diabetic cardiomyopathy

We performed ultrasound, echocardiography studies to further evaluate the efficacy of ET-traps in ameliorating type I diabetes related pathology. Ejection fraction (EF) and fractional shortening (FS) were taken as measures of LV systolic function. Our ET-traps significantly return to basal levels EF and FS in diabetic mice (Fig. 5a, b).

E/A and E'/A' were taken as measures of LV diastolic dysfunction. The ET-traps significantly return to basal levels E/A and E'/A' in diabetic mice (Fig. 6a, b).

Histological analyses of myocardium and glomerulus tissue further show the therapeutic benefit of ET-traps

Diabetes causes focal collagen deposition (green stain) in the myocardium, which was not seen in the control or in the ET-traps treated animals (Fig. 7).

Diabetes causes mesangial expansion (magenta stain) in the glomerulus, which was not seen in the controls and was less in the ET-traps treated animals (Fig. 8).

Discussion

Various studies show a significant increase in ET-1 levels in diabetes compared to normal, control subjects [12, 13]. In this study, we evaluated the therapeutic potential (using the B6 mouse model of type I diabetes) of ET-traps, which potently bind and sequester elevated levels of ET-1.

Earlier work shows increased collagen expression and deposition in diabetic hearts [7]. This is consistent with our findings, where we showed that administration of ET-traps returned Col 4 α 1 expression back to control, non-diabetic levels. Previous research has also reported that in Streptozotocin (STZ) induced diabetic mice, fibronectin mRNA levels were significantly increased compared to controls in the kidney cortex (*p* less than 0.02) [29]. Our results also showed that fibronectin mRNA levels were significantly increased in diabetic mice and this was ameliorated by ET-traps treatment. This again demonstrated the therapeutic benefit of ET-traps. Furthermore, histological analyses in our study showed increased collagen deposition in the myocardium of diabetic mice, which was not seen in the controls or in the ET-traps treated animals.

Cardiovascular diseases are a major cause of death and disability in people with diabetes, accounting for 44% of fatalities in people with type 1 diabetes [30, 31]. It has been suggested that aberrant ECM expression contributes to diabetic cardiomyopathy [32]. Diabetic cardiomyopathy is a type of cardiovascular damage that is found in diabetic patients. It is characterized by myocardial dilation and hypertrophy as well as a decrease in the systolic and diastolic functions of the left ventricle [31].

Fig. 4 There is a significant increase in urinary markers of pathology that is ameliorated in subjects treated with ET-traps. **a-c** Protein expression analyses (ELISA) of urinary markers of pathology in B6 mouse subjects treated with or without ET-traps. Urine albumin, creatinine and the albumin to creatinine ratio were all significantly increased in the urine of diabetic mice at the end of the 8-week study period. This increase was absent in subjects treated with 500 nM ET-traps. Data are presented as means $\pm$ SD. Statistical significance of differences between groups was tested with Student's *t* test. A *p* value of 0.05 or less was considered to be significant. * $p \leq 0.05$ compared to non-diabetic controls, ** $p \leq 0.05$ compared to diabetic subjects, *n* = 6, C = control, D = diabetic, 250 = 250 nM ET-traps, 500 = 500 nM ET-traps

Left ventricular diastolic dysfunction is an early sign of diabetic cardiomyopathy [33]. Mitral inflow patterns (E/A ratio) and mitral annulus velocities (E'/A') were used to assess diastolic dysfunction, as reported previously [26]. E/A and the E'/A' ratios were returned to basal levels with ET-traps treatment of diabetic mice. The diastolic dysfunction is the first abnormality, and with time, leads to systolic dysfunction [34]. In our study, we observed an

improvement in EF and FS in the ET-traps treated diabetic mice.

Analogous to previous research that found increased albumin excreted in the urine in chronic diabetes [35], we observed a significant increase in urine albumin in diabetic mice. This was not seen with ET-traps treatment. Previous research also shows the importance of the identification of albuminuria levels, as well as increased serum creatinine and decreased

Figs. 5 and 6 ET-traps have a positive effect on diabetic cardiomyopathy. We performed ultrasound, echocardiography studies to determine EF, FS, and the E/A and E'/A' ratios. There is a significant decline in all measure of cardio pathology in diabetic mice at the end of the 8-week study period. This decline was absent in subjects treated with 500 nM ET-traps, which exhibited a significant return to control, non-diabetic levels of all markers of cardio pathology. Statistical significance of differences between groups was tested with a Student's t-test or an ANOVA (Fisher's Protected Least Significant Difference test). A p value of 0.05 or less was considered to be significant. * $p \leq 0.05$ compared to non-diabetic controls, ** $p \leq 0.05$ compared to diabetic subjects, $n = 6$, C = control, D = diabetic, 250 = 250 nM ET-traps, 500 = 500 nM ET-traps

Figure 6

haemoglobin levels to predict the development of end-stage renal disease (ESRD) in patients with diabetes and nephropathy [21]. Diabetic nephropathy is the leading cause of end-stage chronic kidney disease (CKD) or ESRD in most developed countries [36]. This is a major problem, as shown by previous research; one study described that in the US alone, the costs for CKD patients older than 65 reached over \$45 billion, which shows the economic burden associated with CKD. Patients with ESRD require lifelong dialysis and the only possible treatment is kidney transplant [37]. The results of our study indicate that ET-traps could help prevent progression to CKD and become a potential therapy for this.

Our results showed increased Col 4 α 1 and FN levels in the kidneys of diabetic mice. Previous work described the expression of extracellular matrix proteins in the kidney as a biomarker of CKD [37]. The urine albumin test or albumin/creatinine ratio (ACR) is used to screen people with chronic conditions, such as diabetes and high blood pressure (hypertension) to test for the development of kidney disease. In our study, we found increased levels of creatinine and albumin in the urine of diabetic subjects. This was significantly reduced in diabetic mice treated with ET-traps, which further

supports the use of ET-traps as a therapeutic tool for type 1 diabetes.

The advantage of our approach over ET antagonists is that we merely (potently) bind and sequester pathologically elevated levels of ET-1, without completely blocking ET-1 function that is essential and therefore, we do not see any side effects. Lowering the dosage of the antagonist (inhibitor drug) or the number of days administered per week, might help mitigate the side effects but a lower dose of the inhibitor drug may mean lower efficacy and therefore less symptom relief. Although this in vivo study looked specifically at type 1 diabetes, our previous in vitro work pertained to both type 1 and type 2 diabetes [10]. Hence, future work can also look at the benefits of our ET-traps in type 2 diabetes in vivo.

Conclusion

In summary, using a mouse model of diabetes, we found a significant therapeutic effect of the ET-traps on different markers of pathology, including ECM expression in the heart and kidneys, systolic function and diastolic dysfunction of the

Fig. 7 Histological analyses of myocardium tissue show the therapeutic benefit of ET-traps. Diabetes causes focal collagen deposition (green stain) in the myocardium, which was not seen in the control or in the ET-traps treated animals, $n = 6$. Bar = 10 µm (same magnification for all micrographs of Fig. 7)

Fig. 8 Histological analyses of glomerulus tissue further show the therapeutic benefit of ET-traps. Diabetes causes mesangial expansion (magenta stain, arrow) in the glomerulus, which was not seen in the controls and was less in the ET-traps treated animals, $n = 6$. Bar = 10 µm (same magnification for all micrographs of Fig. 8)

heart, and urine creatinine and albumin levels. Our results showed that ET-traps potentially and significantly reduced each of these pathologic markers back to control, non-diabetic levels. Given these positive in vivo results and the fact that ET-traps did not exhibit any toxicology, ET-traps may become a new therapeutic tool for type 1 diabetes. Next steps would include further evaluating the use of ET-traps as a therapy for diabetes, including taking them through clinical trials.

Author contributions AJ participated in the research design. The in vivo testing experiments were contracted out as stated at the beginning of the Materials and methods. AJ, AJ and VM contributed new reagents or analytic tools. AJ, VM, IJ and AJ wrote or contributed to the writing of the manuscript.

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Compliance with ethical standards

Ethics approval statement All experiments conformed to the *Guide For the Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH publication no. 85–23, revised in 1996). Experimental protocols were reviewed and approved by the Western University council on animal care committee.

Conflict of interest AJ, VM and AJ are members of Accelerate Cambridge, University of Cambridge, UK.

Declaration statement Any inquiries on data of this study can be directed to Dr. Arjun Jain.

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