Serelaxin treatment reverses vascular dysfunction and cardiomyocyte hypertrophy in the streptozotocin-induced mouse model of Type 1 diabetes

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Introduction: Endothelial dysfunction, cardiomyocyte hypertrophy and interstitial fibrosis are pathological hallmarks of diabetes-related complications. Serelaxin (recombinant human relaxin-2) treatment prevents hyperglycaemia-induced endothelial dysfunction in the mouse aorta *ex vivo*, and limits apoptosis and hypertrophy in neonatal rat cardiomyocytes *in vitro.* Little is known about the effects of *in vivo* serelaxin treatment in a Type 1 model of diabetes. This study aimed to test the hypothesis that serelaxin (RLX) treatment reverses diabetes-induced vascular dysfunction and left ventricular remodelling in streptozotocin (STZ)-injected mice.

Methods: Type 1 diabetes was induced in mice by five consecutive daily intraperitoneal injections of STZ (55 mg/kg body weight, in 0.1 M citrate buffer, pH4.5) with overnight fasting. An equivalent volume of citrate buffer was injected into the mice in control group. Eleven weeks after the initial STZ/citrate buffer injections, the mice were further allocated into citrate buffer+placebo (20 mM sodium acetate, pH5.0), STZ+placebo and STZ+RLX (0.5 mg/kg/day) treated groups. Placebo and RLX treatments were administered *via* sterile subcutaneous osmotic pump for two weeks. Blood glucose levels were monitored fortnightly two weeks after the initial STZ/citrate buffer injections using a glucometer. Mice with blood glucose levels greater than 25 mM were considered diabetic. After 12 weeks of diabetes, the mice were anaesthetized by a cocktail of ketamine (85 mg/kg) and xylazine (8.5 mg/kg) *via* intraperitoneal injections followed by cardiac puncture. Vascular function and left ventricular morphology were assessed using wire myography in the absence or presence of pharmacological blockers and histology, respectively.

Results: After 12 weeks of diabetes, sensitivity to the endothelium-dependent vasodilator, acetylcholine was reduced in the mesenteric artery (pEC50; CB+placebo 7.57±0.11 *vs* STZ+placebo 6.76±0.07, n=12-13, $P<0.0001$). This was accompanied by an increased contribution of vasoconstrictor prostanoids and a decrease in endothelium-derived hyperpolarisation (EDH)-mediated relaxation. Serelaxin restored mesenteric artery endothelial function (pEC50; 7.19±0.12, n=12) by increasing nitric oxide (NO), but not EDH-mediated relaxation. It also normalized the contribution of vasoconstrictor prostanoids and suppressed diabetes-induced hyper-responsiveness to angiotensin II (Ang II). In the left ventricle, diabetes promoted significant hypertrophy (cardiomyocyte cross-sectional area; n=6, $P<0.0001$) and fibrosis (interstitial collagen area; n=6, $P=0.006$), whereby serelaxin effectively reversed cardiomyocyte hypertrophy through a reduction in B-type natriuretic peptide gene expression. Interestingly, serelaxin did not induce any significant effects in attenuating diabetes-induced cardiac fibrosis.

Conclusions: *In vivo* serelaxin treatment for two weeks attenuated diabetes-induced endothelial dysfunction in the mesenteric artery of STZ mice. This was partly attributed to a serelaxin-mediated reduction in the contribution of vasoconstrictor prostanoids and Ang II to vascular function, as well as an up-regulation of NO-mediated relaxation in the mesenteric artery. Favourable effects of serelaxin were also evident in the left ventricle because there was a reduction in diabetes-induced cardiomyocyte hypertrophy. This study suggests a potential role for serelaxin as an adjunctive agent in the treatment of diabetes-related cardiovascular complications.