Heparan sulphate contributes to microvascular permeability and can be therapeutically targeted in diabetes

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Objectives: The endothelial glycocalyx (eGlx) is a protective layer lining the luminal side of blood vessels, containing proteoglycans (core proteins with glycosaminoglycan (GAG) sidechains). In diabetes mellitus (DM), systemic damage to eGlx leads to increased vascular permeability, as observed in diabetic retinopathy and nephropathy. Heparan sulphate (HS) is the most abundant sulphated GAG in the eGlx and heparanase, an HS degrading enzyme, is upregulated during diabetes. We hypothesised that eGlx HS contributes to solute permeability in the retina and glomerulus, and constitutes a therapeutic target to prevent pathological microvascular permeability in DM.

Methods: EGlx HS was depleted in healthy mice by heparinase III (hepIII) treatment, or by knock-down of Ext1 (an HS biosynthesis enzyme) in endothelial cells using Tie2rtTA, tet-O-Cre, $Ext1^{fl/fl}$ ($Ext1^{fl/fl}$) mice. *Db/db* mice (type 2 DM) were administered a heparanase inhibitor (20mg/Kg, i.p. daily, OVZ/HS-1638 (PMID: 30480427)) from 9-11wk of age. Lectin staining and electron microscopy was used to quantify eGlx changes in the eye and kidney, respectively. Fluorescein angiography was used to measure retina solute flux in hepIII-treated and $Ext1^{flx/flx}$ mice and albumin leak was quantified in db/db retinas by immunofluorescence. Glomerular albumin permeability (Ps'alb) and urinary albumin creatinine ratios (uACR) were measured at end point.

Results: In hepIII-treated and $Ext1^{fl/fl}$ mice, eGlx depth was significantly reduced and solute flux was increased across the blood-retina-barrier. Similarly, glomerular eGlx depth was reduced and Ps'alb was increased in both models. In *db/db* mice, eGlx depth was significantly reduced in both the retina and glomerulus compared to control mice (lean) and restored by OVZ/HS-1638 treatment. There was a significant increase in extravascular retinal albumin in *db/db* mice, which was significantly reduced in OVZ/HS-1638 treated mice. Similarly, Ps'alb and uACR was significantly increased in *db/db* mice compared to lean mice but was prevented by OVZ/HS-1638 treatment.

Conclusions: Damage to eGlx HS in the retina and glomerulus resulted in increased solute permeability. Blocking HS degradation during DM restored eGlx in the retina and kidney, and reduced retinal extravascular albumin and glomerular permeability in the kidney. These results suggest HS could be a therapeutic target in diabetes and demonstrate a potential for OVZ/HS-1638 as a therapeutic treatment to prevent both diabetic retinopathy and nephropathy in DM.

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