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A novel heparanase inhibitor protects glomerular endothelial glycocalyx during diabetes mellitus

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An estimated 647 million people worldwide will have diabetes mellitus (DM) by 2040, which causes life altering microvascular complications, like diabetic nephropathy (DN) and retinopathy (DR). The endothelial glycocalyx (eGlx) is a protective layer that lines the luminal side of blood vessels and contains proteoglycans (core proteins with glycosaminoglycan (GAG) sidechains) that help maintain vascular permeability, and are damaged during DM. Heparan sulfate (HS) is the most abundant GAG in the eGlx and heparanase, an HS degrading enzyme, is upregulated during diabetes.[1] The objective of this study is to demonstrate the importance of HS in the eGlx and to show that protection of HS by heparanase inhibition prevents eGlx damage and associated pathological microvascular permeability.

C57BL/6 mice were administered a retro-orbital bolus injection of Heparinase III (HP). Thirty minutes later anaesthetised mice were cardiac perfused with Ringer's solution followed by glutaraldehyde and Alcian blue for electron microscopy (EM). Db/db mice (type 2 DM) were administered a novel heparanase inhibitor (HI) from 9-11wk of age.[2] Similarly, mice were cardiac perfused with Ringer's solution to flush kidneys before using an ex vivo glomerular permeability assay, followed by glutaraldehyde and Alcian blue for EM.[3] Alcian blue perfused kidneys were processed for EM, imaged, and eGlx depth and percent coverage were measured using ImageJ. Glomeruli were isolated from ringer perfused kidneys and apparent albumin permeability (Ps'alb) was measured in single capillaries from individual glomeruli.

Treatment with HP in C57BL/6 mice significantly reduced eGlx coverage in glomerular capillaries by 20%, confirming that targeted degradation of HS leads to eGlx damage. Db/db mice were diabetic and proteinuric and had significant changes to glomerular filtration barrier ultrastructure (quantitative EM). HI significantly reduced Ps'alb in glomeruli isolated from db/db mice by 19.4% compared to db/db controls. EM quantification showed significant increase in eGlx thickness and coverage by 32.9% and 19.78%, respectively. Increased eGlx thickness correlated with decreased Ps'alb.

Results confirmed that degradation of HS causes damage to eGlx in the kidney. Furthermore, inhibition of heparanase during DM restored eGlx structure and function during DN, suggesting potential for this novel HI as a therapeutic treatment during DM microvascular complications. Using a new class of HI, future work will investigate HS in the retinal eGlx and its contribution to vascular permeability in addition to kidney eGlx.