Endothelial heparan sulphate depletion leads to basement membrane thickening

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Abstract

In diabetes, basement membrane (BM) thickening in the retina and glomerulus is associated with increased permeability and, in the case of the kidney, proteinuria. The endothelial glycocalyx (eGlx) is the primary macromolecular filter within the vascular wall. This pilot study tests if eGlx disruption leads to the BM phenotype observed in diabetes.

Heparan sulphate (HS) is a ubiquitous glycosaminoglycan chain within the extracellular matrix. HS biosynthesis involves polymerisation mediated by glycosyltransferases, with the product of the Ext-1 gene essential for HS elongation. Homozygous inducible Ext-1 knockout (KO) mice were used in this study, where the Cre recombinase used to initiate the KO is under the control of the VE-cadherin promoter restricting the KO to the endothelium. Here we present our initial findings from a pilot study (n=2 per group).

VeCadCre$^{+/-}$ mice were given a daily intraperitoneal dose of 1mg tamoxifen per 25g mouse to induce KO or sunflower vehicle for 5 days. A further littermate control group, not expressing Cre recombinase (Ext-1$^{fl-/fl-}$ VeCadCre$^{-/-}$), was treated with tamoxifen.

Fundus fluorescein angiography (FFA) was performed on days 0, 22, 36 and 57 following induction of the KO to calculate retinal permeability. On day 59, one kidney was removed from each mouse under terminal anaesthetic (75 mg/kg Ketamine and 1mg/kg Medetomidine) and used to determine glomerular permeability. The mice were then perfusion fixed with the LaDy GAGa technique[1], and the tissues were removed, stained and embedded in resin. The samples were then sectioned, imaged with Transmission Electron Microscopy (TEM) and the basement membrane thickness measured using FIJI software.

eGlx coverage was reduced in the Ext-1$^{fl-/fl-}$ VeCadCre$^{+/-}$ tamoxifen-induced group, with an increase in retinal BM thickness (0.30 ± 0.07 mean ± SEM) compared with vehicle controls (0.12 ± 0.01; nominal $p < 0.05$) and VeCadCre negative (0.14 ± 0.05; ns) littermate controls. This is consistent with observed diabetic BM alterations. However, there was no significant difference in the glomerular or retinal permeability, possibly due to the small cohort size.

The results suggest that endothelial heparan sulphate reduction could be one of the factors responsible for the reversible BM changes observed in diabetes. However, the mechanism has yet to be elucidated, although these observations are consistent with the volumetric expansion hydration hypotheses rather than deposition of new material in diabetic BM thickening.

1. Arkill, K.P., et al., *3D reconstruction of the glycocalyx structure in mammalian capillaries using electron tomography.* Microcirculation, 2012. **19**(4): p. 343-351.