Heparin infusions for enhanced glomerular barrier function in diabetic nephropathy

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Diabetic nephropathy (DN) is the leading cause of end stage renal failure in the western world. Current therapies focus on lowering blood pressure or managing insulin, but additional novel targets are urgently sought. Damage to the protective endothelial glycocalyx (e-GLX) lining of the glomerular filtration barrier, the site of ultrafiltration in the kidney, occurs early in the development of DN. Previously Sulodexide, a mixture of heparin sulphate (HS) and dermatan sulphate, has been shown to protect the e-GLX and reduce albuminuria in DN patients. There is evidence for [1-3] and against [4, 5] clinical continuation in DN, although it is used in other vascular diseases [6-9]. Taking a similar approach, but using heparin infusions, we aim to show direct binding to the e-GLX and a reduction in albuminuria.

Heparin will be tested for its ability to bind the e-GLX directly on glomerular endothelial cells (GEnC) in culture. Once confirmed it will be perfused into the kidneys of diabetic or non-diabetic mice via the abdominal aorta under terminal anaesthesia. Glomeruli will be isolated, heparin binding to e-GLX imaged and albumin permeability measured using a novel confocal microscopy assay. Heparin will then be given over a two week period to Type I (streptozotocin) and Type II (db/db) diabetic mice at a time point when albuminuria is known to develop. Urine/albumin creatinine ratios will be analysed from these mice. At termination, one kidney will be removed for immunofluorescence to demonstrate heparin glomerular e-GLX binding, the other will be perfusion fixed with glutaraldehyde containing Alcian blue to visualise and measure the e-GLX by quantitative electron microscopy.

This project will use tissue culture, *ex vivo* assays and animal models to examine the therapeutic potential of heparin infusion in DN. Together these will demonstrate whether heparin binds directly to the glomerular e-GLX, increases e-GLX thickness/coverage and improves glomerular barrier function.

References

1. Li, R., et al., *Sulodexide therapy for the treatment of diabetic nephropathy, a meta-analysis and literature review.* Drug Des Devel Ther, 2015. **9**: p. 6275-83.
2. Zilisteanu, D.S., T. Atasie, and M. Voiculescu, *Efficacy of long-term low-dose sulodexide in diabetic and non-diabetic nephropathies.* Rom J Intern Med, 2015. **53**(2): p. 161-9.
3. Coccheri, S., *Game not over for sulodexide.* Am J Kidney Dis, 2012. **59**(3): p. 467.
4. Gambaro, G., *Discounting the efficacy of sulodexide in diabetic nephropathy is premature.* Am J Kidney Dis, 2012. **60**(1): p. 169-70.
5. Lewis, E.J., et al., *Sulodexide for kidney protection in type 2 diabetes patients with microalbuminuria: a randomized controlled trial.* Am J Kidney Dis, 2011. **58**(5): p. 729-36.
6. Elleuch, N., et al., *Sulodexide in Patients with Chronic Venous Disease of the Lower Limbs: Clinical Efficacy and Impact on Quality of Life.* Adv Ther, 2016. **33**(9): p. 1536-49.

7. Andreozzi, G.M., et al., *Sulodexide for the Prevention of Recurrent Venous Thromboembolism: The Sulodexide in Secondary Prevention of Recurrent Deep Vein Thrombosis (SURVET) Study: A Multicenter, Randomized, Double-Blind, Placebo-Controlled Trial.* Circulation, 2015. **132**(20): p. 1891-7.
8. Song, J.H., et al., *Effect of sulodexide in patients with non-proliferative diabetic retinopathy: diabetic retinopathy sulodexide study (DRESS).* Graefes Arch Clin Exp Ophthalmol, 2015. **253**(6): p. 829-37.
9. Coccheri, S. and F. Mannello, *Development and use of sulodexide in vascular diseases: implications for treatment.* Drug Des Devel Ther, 2013. **8**: p. 49-65.