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Science Abstracts

SA1. Recombinant Complement Factor H in a Model of C3 glomerulopathy

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BACKGROUND: C3 glomerulopathy is an inflammatory renal disorder that is associated with abnormal complement alternative pathway activation. This includes deficiency of complement factor H (FH), the negative regulator of the alternative pathway. FH gene-targeted ($FH^{-/-}$) mice are used as a model of C3 glomerulopathy and spontaneously develop reduced plasma C3 levels and abnormal deposition of C3 within the glomerulus. We tested the efficacy of recombinant murine FH (mrFH) to restore C3 regulation in $FH^{-/-}$ mice.

METHODS: MrFH was produced in *Pichia pastoris.* Plasma-purified murine FH (mFH) or human recombinant FH (hrFH) was used as a control. In experiment 1, mice received an injection of mrFH or mFH and were culled at 24 hours. In experiment 2, mice received a daily injection of mrFH or hrFH for 10 days and were culled at 11 days.

RESULTS: A single injection of mrFH resulted in increased plasma mrFH and C3 levels peaking at six hours. In mice receiving mFH, plasma FH and C3 levels remain elevated at 24 h. Glomerular histology at 24 hours showed a significant decrease in glomerular C3 staining both for mice receiving mFH and mrFH. In experiment 2, mice receiving daily injections of either mrFH or hrFH exhibited elevated levels of both plasma FH and C3 at 24 hours, but these decreased to baseline within five days. All mice showed reduced C3 glomerular staining at 11 days. Mice receiving hrFH showed strong glomerular IgG staining at 11 days.

CONCLUSIONS: Restoration of complement regulation is the goal of treatment for C3 glomerulopathy. In our experiments mrFH increased plasma C3 and FH levels and reduced glomerular C3 deposition. The rapid reduction in FH and C3 levels (relative to a slower decline in mice treated with plasma-purified FH) may be due to glycosylation differences between plasma-purified and our recombinant protein. Administration of recombinant FH is a rational treatment choice for patients with C3 glomerulopathy associated with deficiency or dysfunction of FH. However, the challenges of this approach include plasma half-life, immunogenicity and large scale production.

Conflict of Interest: none

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SA2. Urinary peptidomics in a rodent model of diabetic nephropathy highlights epidermal growth factor as a biomarker for renal deterioration in patients with type 2 diabetes

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Albuminuria is the gold standard diagnostic and prognostic urinary biomarker for nephropathy in patients with diabetes, however many patients with declining renal function remain normoalbuminuric. To identify alternative biomarkers we performed urinary peptidomic analysis in a novel rodent model of diabetic nephropathy (DN) in which type 1 diabetes is induced by injection with streptozotocin and renin-dependent hypertension is concurrently induced by expressing murine renin cDNA. The severe hyperglycaemia and hypertension synergise such that after 28wks the rodents demonstrate clinical and renal pathological and transcriptomic features consistent with human DN.

Analysis of the urinary peptidome identified 297 increased and 15 decreased peptides in the urine of DN rats compared with controls, including peptides derived from proteins previously known to be associated with DN and novel candidate biomarkers. We confirmed by ELISA that one of the parent proteins, epidermal growth factor (EGF), was more than x2-fold reduced in the urine of DN rats in comparison with controls. To assess the clinical utility of urinary EGF (uEGF) we examined renal outcomes in 642 participants from the Edinburgh Type 2 Diabetes study (ET2DS) who were normoalbuminuric and had preserved renal function at baseline. A lower uEGF:creatinine ratio was associated with new-onset eGFR <60 ml/min/1.73m^2, rapid (>5% per annum) decline in renal function or the composite of both outcomes (OR 0.50; 95%CI 0.34-0.75; p=0.001). These associations were independent of established clinical risk factors for DN.

The utility of low uEGF concentration as a biomarker of progressive decline in renal function in normoalbuminuric patients with diabetes should be assessed in additional populations.

SA3. Nox4 Inhibition, by GKT 137831, Decreases Obesity-Associated Vascular Dysfunction and Pro-Fibrotic Effects.

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The pathophysiological role of Nox4 remains elusive and controversial. Even though Nox4 expression is increased in hypertension, diabetes and obesity, some studies demonstrate that Nox4 deficiency predisposes to obesity and insulin resistance. We showed that Nox1/4 inhibition is renoprotective in diabetes. Whether Nox4 influences vascular function and kidney status in obesity associated with mild diabetes is unclear. We hypothesized that Nox4-specific inhibition is protective against obesity-associated vascular and kidney damage. We studied db/m (control) and db/db mice (obese) mice for 16 weeks receiving 1) vehicle; 2) low dose Nox4 inhibitor, GKT137831 (GKT) (20 mg) and 3) high dose GKT (60 mg) for 16 weeks. Body weight increased in db/db (61.8g ± 0.95) versus controls (33.5g ± 0.72, $p<0.05$). Plasma glucose and albuminuria were slightly increased in db/db. GKT did not influence body weight but reduced epididymal fat mass in db/db (20% increase, $p<0.05$). Blood pressure was similar in all groups. Kidney weight (25%) and markers of renal fibrosis, such as, fibronectin (60%), pro-collagen I (PCI - 50%) and TGFβ (30%), were increased in db/db versus controls ($p<0.05$). High dose GKT decreased renal expression of fibronectin and PCI in db/db. Renal ERK1/2 activation was ameliorated by high dose GKT in db/db (vehicle, 65% vs GKT, 2.3%, $p<0.05$). Plasma 8-isoprostanes (db/db: 1263±96 vs db/m: 936±35), marker of systemic oxidative stress, and kidney hydrogen peroxide (db/db: 1 fold increase vs db/m) levels were increased in db/db, effects blocked by GKT. Endothelial dysfunction in db/db mice was not affected by GKT, but decreased NE-induced vascular contraction in db/db (pD2: db/db vs. db/db high dose, 6.4±0.1 vs. 5.6 ± 0.1, $p<0.001$). Reduced contraction by GKT was associated with decreased Rho kinase activity (db/db: 84% increase vs db/db+GKT 60 mg: 29% increase, $p<0.05$). Our findings suggest a role for Nox4 in obesity-associated kidney and vascular damage/dysfunction, important factors predisposing to cardiovascular disease in obesity. Mechanisms underlying Nox4 effects involve oxidative stress and Rho kinase.

SA4. Molecular markers of biological ageing in Delayed Graft Function

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Delayed graft function is a prevalent clinical problem in renal transplantation for which there is no objective system to predict in advance. It can result in a significant increase in the necessity for hospitalisation post-transplant and is a risk factor for other post-transplant complications. In our recent studies, we have identified a pre-transplant signature panel of microRNAs, which can be related to post-transplant renal allograft performance as well as adverse outcomes. Here we demonstrate, in two independent cohorts of pre-implantation human renal allograft biopsies, that a novel pre-transplant renal performance scoring system (GRPSS), can determine the occurrence of DGF with a high sensitivity (>90%) and specificity (>60%) for donor allografts pre-transplant, using just three senescence associated microRNAs combined with donor age and type of organ donation. Additionally, this signature has the ability to discriminate between DCD and DBD donor organs.

We believe that the GRPSS will provide clinicians with a simple, rapid quantitative molecular pre-transplant assessment of donor allografts to determine post-transplant allograft function in real time as the organ is being cross matched. Furthermore, this study demonstrates the involvement of senescence pathways in ischaemic injury during the organ transplantation process and highlights accelerated organ ageing as a consequence of both warm and cold ischaemia, with a direct consequence of subsequent long term organ performance.

SA5. The impact of a young circulation on renal injury and fibrosis in aged mice

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Introduction

Aging is associated with an increased risk of acute kidney injury (AKI) and higher rates of subsequent fibrotic chronic kidney disease (CKD). The mechanisms accounting for these changes in injury and fibrosis susceptibility remain poorly understood. Recent studies using heterochronic parabiosis have shown that in other organ systems (brain/heart/muscle) exposure to a young circulation can reverse aging associated phenotypes. The effects of parabiosis on injury outcomes in the aged kidney remain unknown.

Materials and Methods

Parabiotic pairs were established between young (Y, 8 weeks old) and old (O, 14 months old) female C57BL6 mice to generate YY, OO, OY and YO pairings, and a shared microcirculation was allowed to develop for 28 days. Baseline renal characteristics were assessed by immunofluorescence, western blotting and microarray analysis. Acute injury was induced via 20 minutes bilateral renal ischaemia reperfusion injury, and chronic fibrosis via unilateral ureteric obstruction. Animals were followed for 24hrs for peak IRI severity, and for up to 14 days for UUO induced fibrosis

Results

Baseline renal function was equivalent in all groups assessed. After 28 days of parabiosis, old animals in both OO and OY pairings had increased levels of baseline fibrosis ($p<0.05$) compared to the young in YO and YY pairs. Significant reductions were seen in levels of senescent renal cells assessed by p16INK4a positivity in OY, YO and YY when compared to OO animals ($p<0.05$ OO vs other groups). Microarray studies demonstrated over 1000 genes associated with the ageing process are altered in the baseline kidneys of old animals in OO pairs. Over 100 of these revert to a 'young' phenotype in OY pairings, with 15/59 highest fold-change genes reverting to a young baseline in Old animals in OY pairs c/w OO animals. In the acute injury model, OY animals demonstrate significantly lower d1 serum creatinine compared with OO animals ($p<0.05$). In the UUO model of chronic renal fibrosis, Old animals in OY pairs have reductions in scarring, senescent cell number, macrophage infiltration and inflammatory gene transcription compared to OO pairs (all $p<0.05$).

Conclusions

Our data shows that the normal aged kidney exhibits differences from the young kidney in levels of cellular senescence and at a transcriptomic level even with well maintained renal function. Furthermore, connection to a young circulation modifies the transcriptional signature in the aged baseline kidney and protects against subsequent AKI and progressive renal scarring, indicating the presence of a circulating factor, which modifies renal aging. Further proteomic analysis of serum samples and microarray interrogation of the injured kidney is ongoing to identify the causative factor(s) involved.