Determining albumin degradation rates in the proximal tubule

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Albuminuria is a marker of renal impairment and predicts poor cardiovascular and renal outcome in subjects with diabetes and hypertension. Albuminuria in itself is now recognised as a major mediator of tubular damage contributing to the progression of renal fibrosis and chronic kidney disease. Significant controversy persists around how albumin is handled by the kidney and how these pathways are affected during renal disease. Currently, there is no consensus as to how much albumin is processed by the proximal tubule and how many pathways are involved. However, it is widely accepted that a significant proportion of filtered albumin is endocytosed by the proximal tubule and undergoes proteolytic degradation to constituent amino acids in the proximal tubular epithelial cells (PTECs) to be reutilised by the body. Much research has focused on the molecular mechanisms underlying receptor-mediated albumin endocytosis. While this is of obvious importance, the other side of albumin processing in the proximal tubule, *i.e.,* degradation remains largely unexplored. Little experimental data exists regarding the dynamics of albumin degradation in the proximal tubule and how this pathway is affected by pathophysiological conditions or disease. This lack of data can be attributed to the difficulty in accurately and quantitatively assessing albumin degradation in a robust manner.

In this study, we have assessed the suitability of two auto-quenching fluorescent albumin conjugates, DQ-green-BSA and DQ-red-BSA as experimental tools to correlate the degradation of albumin with it's uptake in the proximal tubule both *in vitro* and *in vivo*. Initially, the characteristics of the DQ-BSA probes were assessed. In their native forms, DQ-BSA probes did not fluoresce above background levels. Fluorescence was detected after tryptic digestion of the probes in a time- and concentration- dependent manner. The probes were stable across a range of pHs, again only fluorescing after tryptic digest. Using a proximal tubular epithelial cell line (OK cells), albumin degradation in live cells was assessed. Cells were incubated with increasing amounts of DQ-BSA over a 3 hour timecourse. Fluorescence was detected in cell lysates in a time- and concentration-dependent manner. DQ-BSA fluorescence was reduced by the addition of increasing concentrations of unlabelled BSA, demonstrating the specificity of the pathway involved. The rate of albumin degradation was seen to be proportional to its uptake and at any given time point, approximately 40-50% of albumin had been degraded. Inhibition of the lysosomes with CHQ resulted in significant inhibition of albumin degradation in cells. Similarly, treatment of cells with an inhibitor of receptor mediated endocytosis, latrunculin A also significantly reduced albumin degradation. Confocal analysis revealed that DQ-BSA fluorescence was localised in large vesicles concentrated towards the baso-lateral surface of the PTECs. Together, these results indicated that DQ-BSA fluorescence was dependent on a receptor-mediated, lysosomal degradation pathway that had high affinity for albumin. This was also examined *in vivo*. Normal wistar rats were injected with 2mg DQ-BSA intra venously and sacrificed at 5, 10 and 20 minutes after injection. Kidneys were removed and fixed for sectioning. DQ-BSA fluorescence indicating albumin degradation in PTECs was evident at all timepoints examined. Further studies were performed to determine if albumin degradation was affected by pathophysiological conditions associated with CKD. In OK cells, albumin degradation was significantly decreased by pre-exposure to TGF-β1, high glucose and angiotensin II.

Together, these results have validated DQ-BSA probes as tools to analyse albumin degradation in the proximal tubular cells allowing correlation of albumin uptake and degradation showing that ~40-50% of reabsorbed albumin is being degraded at any given time. Further studies will allow us to delineate more fully the components of albumin uptake pathway and examine how this degradation pathway is modulated under disease conditions.