MRI Biomarkers for Monitoring Progression in CKD: Preliminary Experience in a Reversible UUO Mouse Model

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Introduction:

The realities of effective but limited resources heighten the need to identify and target patients at highest risk for whom aggressive medical management would have the most impact. In the clinical setting nephrologists are routinely challenged to assess the severity of chronic kidney disease (CKD) in patients and their risk for progression. Current clinical markers such as serum creatinine, kidney size, and proteinuria are not sufficient to reliably detect early CKD or predict risk of progression. Kidney biopsy can provide useful information but is invasive and poses inherent risks and can be subject to sampling error issues. Hence, it is highly desirable to develop new, non-invasive markers that could identify those subjects with CKD, particularly in early stages, and those that will progress to end stage renal disease.

In this preliminary study, we investigated the use of apparent diffusion coefficient (ADC) as a measure of fibrosis and BOLD MRI to detect early changes in oxygenation status. While these proposed MRI biomarkers are readily applicable to humans, the fact that the progression of CKD is slow and happens over years or decades does not allow for testing feasibility and validation. Pre-clinical models allow for a more manageable time frame to monitor progression and validation against conventional standards such as histology. Rodent models are cost-effective and mouse models allow for studying genetic variations.

Unilateral ureteral obstruction (UUO) is a well-described model of renal fibrosis and is considered a model of CKD (*Klahr S et al AJP Renal Physiol 2002*). However, this model does not allow study of longitudinal changes because the renal parenchyma is lost completely in few weeks (Fig. 1). An innovative model system of CKD using reversible ureteral obstruction (rUUO) that allows for functional assessment of renal function after injury in parallel with structural and molecular studies was recently described (*Puri et al AJP Renal Physiol 2010*). Using this model, it was identified that inbred strains of mice which are either susceptible (C57BL/6) or resistant (BALB/c) to development of CKD after rUUO mediated injury. Susceptible mice demonstrate loss of renal function and the development of tubulointerstitial fibrosis after injury (*Puri et al AJP Renal Physiol 2010*), which is considered to be a hallmark of development and progression of CKD.

Material and Methods:

All surgical procedures were performed in compliance with our institutional animal care guidelines. A total of 20 mice, ten each of C57BL/6J and BALB/C, under went rUUO surgical procedure as previously described (*Puri et al AJP Renal Physiol 2010*). The right ureter was ligated using microvascular clips (S & T Inc., Foster City, CA). The clip was moved distally every 2 days and removed on the 6th day. Approximately after one week of recovery period the left ureter was obstructed permanently to allow for monitoring functional changes due to rUUO in the right kidney. MRI was performed at three time points *viz.* baseline, day-2 and day-30 following the removal of the ureteral clip on the right kidney. A 4.7T (Bruker Biospec, Billerica, MA) system with Avance II and a dedicated mouse body coil (Rapid MR International, Columbus, OH) were used to acquire MRI data. BOLD imaging parameters include: Pulse Sequence= MGE, FOV = 4 x 4mm, No. of Slice = 1, Slice thickness = 0.75mm, Matrix = 256 x 256, TR = 50ms, No Echo = 8 equally space (3.21 – 34.3ms). NEX = 20. DWI: Pulse Sequence = DWI_EPI, FOV = 4 x 4, No. Slice = 1, Slice Thickness = 0.75mm, Matrix = 128 x 128, TR = 3800ms, TE = 31.7ms, No. of Segment = 8, Big delta = 20ms, little delta = 4ms, b-value = 50, 150, 300, 500 mm2/sec. At the end of the study, animals were euthanized and kidneys harvested for histological analysis.

Data Analysis: Three ROIs were selected in the cortical region using Bruker PV5.0 analysis software. A two tail Students t-test was performed to test for statistical significance.

Results and Discussion:

Figure 1 illustrates the changes in the anatomy of the two kidneys subject to rUUO (right) and 4 weeks of permanent ligation (left).

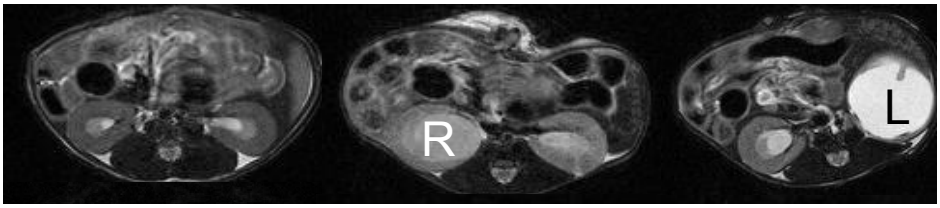
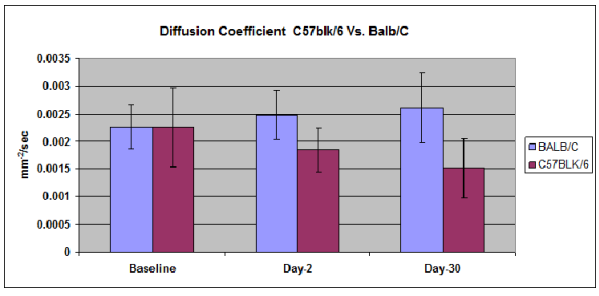


Fig 1: *Axial MR images obtained in a single animal at 3 different time points: baseline, 2 and 28 days after release of obstruction on the right kidney. Consistent with literature (Cochrane AL et al J.Am.Soc.Neph 2005), we see a return of the anatomical structure of the right kidney four weeks following rUUO. In contrast the renal parenchyma is completely lost three weeks after permanent ligation.*

Two day post rUUO imaging showed no distinct cortico-medullary contrast. So we only report on cortical measurements here. There was a progressive decrease in ADC values in C57BL/6 mice which was not present in the BALB/C mice (Fig. 2.a). These observations are consistent with the previous experience with this model showing less fibrosis in the BALB/C (*Puri et al AJP Renal Physiol 2010*).

Baseline BOLD imaging showed higher R2* in BALB/C (47.01 ± 5.23) as compared to the C57BL/6J (39.17 ± 5.4) p value < 0.05. As seen in Fig. 2.b, BALB/C showed a decrease in R2* (suggesting improved oxygenation) at days 2 and 30. On the other hand, C57BL/6 showed little change at day 2 and only day 30 showed a decrease in R2*. The minimal change at early time point may explain the increased susceptibility to developing fibrosis and hence progression of CKD. Further longitudinal studies are necessary to validate this.



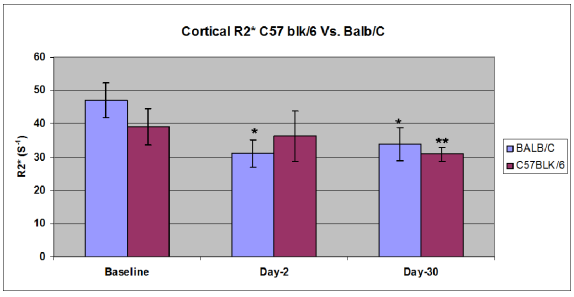


Fig. 11: *(a) summarizes the renal cortical ADC measurements in the two strains obtained at three different time points, baseline, 2, 30 days after rUUO. (b) summarizes the cortical R2* measurements at these three time points.*

Histology showed lower fibrosis scores in BALB/C (1.5 *vs.* 0) and BUN showed statistically significant elevation in both strains but the magnitude of change was higher in the C57BL/6 mice at day 30 (28.6 (bas) – 53.9 (day 30) *vs.* 27.7(bas) – 36.5 (day 30) mg/dl) consistent with previous report (*Puri et al AJP Renal Physiol 2010*).

In conclusion, our preliminary experience with ADC and R2* measurements in rUUO model are consistent with previous reports supporting the utility of these measures as non-invasive imaging biomarkers of disease progression in CKD. Further studies with larger numbers and longer follow up period are warranted.