Longitudinal assessment of renal fibrosis by diffusion weighted MRI: A study in unilateral ureteral obstruction (UUO) mice model

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Introduction: Renal fibrosis is the final common pathology of most progressive renal disease such as diabetic nephropathy and glomerulonephritis. Renal fibrosis has been widely investigated in an established animal model with unilateral ureteral obstruction (UUO) [1]. A number of histopathological studies reported that the progression of renal fibrosis observed in this animal model is similar to that in human: inflammatory cell infiltration, tubular proliferation, epithelial mesenchymal transition (EMT), myofibrobrasts accumulation, increased extracelluar matrix deposition, and then tubular atrophy [1]. In the present study, we investigated the progression of the renal fibrosis in the animal model longitudinally by T2-weighted imaging (T2WI) and diffusion weighted imaging (DWI) to test whether MRI could provide metrics for detection and evaluation of the severity of renal fibrosis.

Material and Methods: *Animal preparation*: After the pre imaging session addressed bellow, UUO was performed on three 8-week old normal mice. Briefly, the right ureters were exposed and permanently ligated by 4-0 nylon sutures at the midline abdominal incision under anesthesia.

MRI: MRI was conducted with a 7T small animal MR scanner (Varian, Inc, Palo Alto, CA) with a 38 mm birdcage RF coil. The animals underwent UUO were placed supine with the respiratory sensor, head first with the abdomen centered with respect to the center of a RF coil. Low-resolution multi-slice imaging, serving as the localizer, was first performed on the abdominal region to confirm the location and orientation of the kidneys. For volume measurement, T2 weighted multi-slice axial images encompassing both ipsilateral and contralateral kidneys were obtained with a fast spin echo sequence (TR/TE = 2500/40 ms, 35 mm FOV, 128×128 matrix, 1 mm slice thickness, gapless, 1 NEX). On the obtained T2WI, volume of the renal parenchyma was measured using an image analyze software (ImageJ). On single 1 mm coronal slab delineating both kidneys (Fig.1), DWI was conducted by using a conventional spin-echo sequence with motion-sensitive gradients parallel to the long axis of the kidneys. Diffusion gradients were applied (Δ = 15 ms, σ = 6 ms) with four b-values of 350, 600, 800, and 1200 s/mm^2. Other parameters were: TR/TE = 3000/27.13 ms, 35 mm FOV, 64×64 matrix, 1 NEX. Respiratory gating was applied to minimize motion artifacts. ADC maps were generated pixel by pixel with the same software (ImageJ) by fitting to the function, $S_1=S_2 \times \exp(-b \times ADC)$, where S_1 and S_2 are signal intensities at different b-values, respectively. ADC was measured in the renal parenchyma on five regions-of-interests (ROIs, typical size = 5.5 mm^2) in each kidney, and an average value of 3 ADCs (excluding a maximum and minimal values) was defined as the representative of the kidney. The same MRI measurements were repeated before and 3 and 7 days (day3 and day7) after UUO in each animal. Values are expressed as mean ± standard deviation (SD).

Histopathology: The mice were sacrificed within 6 hours after the last MR session at day7. Interstitial cellular proliferation and myofibroblast proliferation were evaluated by hematoxylin and eosin (HE) staining and α-SMA staining, respectively.

Results and Discussion: On the ipsilateral kidney, marked dilatation of the renal pelvis was observed at day3 and it deteriorated at day7 while there was no obvious change in the contralateral side through the experiment (Fig. 1A). The volume of the renal parenchyma on the ipsirateral side decreased at day7 (pre: 186.1 ± 9.6, day3: 187.1 ± 8.8, day7: 169.8 ± 12.6 mm^3). By contrast, it tended to increase by time on the contralateral side (pre: 181.6 ± 11.7, day3: 199.0 ± 4.5, 207.0 ± 16.0 mm^3). There was statistical significance between the sides at day7 ($p<0.05$). The mean renal ADCs (10^{-3} mm^2/s) on the ipsilateral / contralateral sides (Fig.1A and 2) were: 0.84 ± 0.07 / 0.84 ± 0.05 (ns) before UUO, 0.65 ± 0.06 / 0.78 ± 0.02 ($p<0.05$) at day3, 0.54 ± 0.03 / 0.84 ± 0.1 ($p<0.01$) at day7. In the histopathologic evaluation, HE staining showed marked increase of cellular density due to cellular proliferation in the interstitial space only in the ipsilateral kidneys in all animalls. α-SMA stains proved this newly proliferated cells were myofibloblasts in the ipsilateral kidneys, which was not observed in the contralateral kidneys.

At UUO side, MRI demonstrated the marked dilatation of the renal pelvis, which results from obstruction of urinary flow. Although this dilatation in the pelivis deteriorated by time, the volume of the renal parenchyma did not change at day3 and then, decreased at day7. However, the parenchymal ADC dropped rapidly approximately 30% at day3 and kept dropping by day7 (42%). We postulate that the reduction of the ADC reflected the increase of cellular density [2] due to the fibroblasts proliferation as observed in the histology. By establishing this animal model with knowledge of renal fibrosis, we may also be able to screen potential drugs for preventing the progression in early stage of the renal disorder.

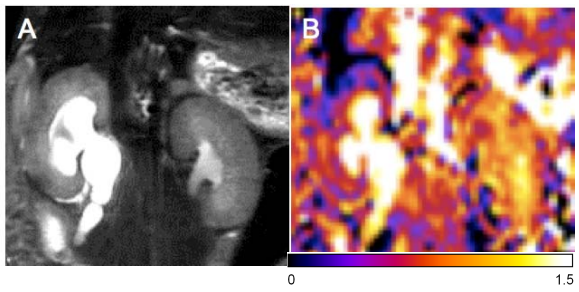

Fig. 1. T2-weighted coronal image of the kidneys 7 days after UUO shows severe hydronephrosis on the ipsilateral side (A). ADC map (B) shows the remarkable ADC decrease on the ipsilateral side compared with that on the contralateral side.

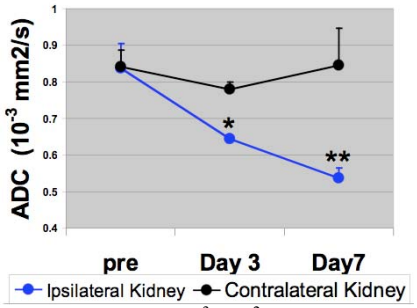

Fig. 2. ADC values (10^{-3} mm^2/s) of the UUO side (ipsilateral) and contralateral kidneys. Values are expressed as mean ± SD. *p<.05, **p<.01 by t-test.

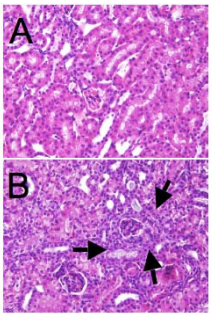

Fig. 3. Histopatholgic specimen of contralateral (A) and ipsilateral (B) kidneys. Marked fibroblasts proliferation in the interstitial space is seen in the ipsilateral specimen (arrows).

Conclusion: MRI, in particular DWI, could sensitively detect and monitor the anatomical and physiological changes in the progression of renal fibrosis in the UUO mice model. ADC potentially serves as a non-invasive maker for evaluating progression of renal fibrosis.

References: 1. Bascands and Schanstra. Kidney Int. 68:925-937(2005). 2. Lyng H et al. Magn Reson Med. 43:828-36 (2000).