

Evaluation of therapeutic effect on renal fibrosis by diffusion-weighted imaging

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Introduction: Chronic kidney disease (CKD) is a worldwide health care problem associated with extensive morbidity and mortality and increasing health costs. The progression of CKD is largely due to progressive fibrosis which is a pathological process characterized by infiltration and proliferation of mesenchymal cells in interstitial space (1). In an experimental murine model of unilateral ureteral obstruction (UUO), we previously demonstrated that diffusion-weighted imaging (DWI) can depict and enable monitoring of abnormal changes in the progression of renal fibrosis (2). A progressive decrease in ADC was observed in the obstructed kidneys only, which correlated with an increase in cell density in the interstitial space, expression levels of α -smooth muscle actin (α SMA), a marker for myofibroblasts, collagen deposition, and tubular atrophy on histologic sections. In the present study, we investigated whether DWI can be used for evaluation of the therapeutic response on the progression of the renal fibrosis in the animal model.

Material and Methods: The klotho gene was identified as a putative aging-suppressor gene in mice that extended life span when over-expressed and induced complex phenotypes resembling human premature-aging syndromes when disrupted. The klotho gene encodes a single-pass transmembrane protein expressed predominantly in renal tubular epithelial cells (3). After the pre imaging session, ten 8-week old mice were underwent UUO and were treated with intraperitoneal injection of Klotho protein (0.02 mg/kg; N=6) or vehicle (N=4) every other day for 7 days.

MRI was conducted in a 7T small animal MR scanner (Varian, Inc, Palo Alto, CA) with a 38 mm birdcage RF coil before, 3 days and 7 days (day3 and day7) after UUO. All animals were placed supine with the respiratory sensor, head first with the abdomen centered with respect to the center of a RF coil. After the localizer imaging, T2W multi-slice axial images encompassing both ipsilateral and contralateral kidneys were obtained with a fast spin echo sequence (TR/TE = 2500/40 ms, 35² mm FOV, 128² matrix, 1 mm slab, gapless, 1 NEX) to measure volume of the renal pelvis and parenchyma. On single 1 mm coronal slab delineating both kidneys, motion-sensitive gradients were applied (Δ = 20 msec, σ = 5 msec) with 5 different b-values of 350, 600, 800, 1000 and 1200 sec/mm² on three orthogonal directions. Other parameters were: TR/TE=3000/38 msec, 30²mm FOV, 64² matrix; 1 mm slab, gapless, 2 NEX, 16 EPI factors, fat suppression. Respiratory gating was applied to minimize motion artifacts.

ADC maps were generated pixel by pixel in each direction by fitting to the function, $S1=S2 \times \exp(-b \times \text{ADC})$, where S1 and S2 are signal intensities at different b-values, respectively. Subsequently, three ADC maps in each gradient direction were averaged for each animal. ADC was measured in the renal parenchyma on five regions-of-interests (ROIs, typical size = 5.5 mm²) in each kidney, and an average value of 3 ADCs (excluding a maximum and minimal values) was defined. The mice were sacrificed within 6 hours after the last MR session at day7 and evaluated interstitial cellular/myofibroblast proliferation, α -SMA and Collagen-1.

Results and Discussion: The average volume of pelvis immediately increased after UUO but was not different between Klotho-treated and vehicle-treated mice, indicating that Klotho treatment did not affect the severity of hydronephrosis induced by UUO. In contrast, the average volume of renal parenchyma in vehicle-treated mice was significantly smaller than that in Klotho-treated mice, suggesting that Klotho treatment prevented parenchymal contraction associated with fibrosis. The average volumes of pelvis and parenchyma in contralateral side did not change in both groups through the protocol. The average ADC (10⁻³ mm²/s) in UUO side progressively reduced in both Klotho and vehicle-treated mice. However, the ADC in Klotho-treated mice was significantly higher than that in vehicle-treated mice: 1.08 \pm 0.04 / 1.07 \pm 0.08 (ns) before UUO, 0.89 \pm 0.10 / 0.81 \pm 0.10 (ns) at day3, 0.85 \pm 0.09 / 0.70 \pm 0.04 (p<0.05) at day7 (Fig. 1A, B), indicating that Klotho treatment alleviated increases in cell density in renal parenchyma. Consistent with the MRI findings, histological analysis confirmed that Klotho protein attenuated increases in cell density and fibrotic tissue (Fig. 2). These observations suggest that Klotho protein injection is an effective treatment for renal fibrosis induced by UUO. We observed significant increase in expression of multiple mesenchymal markers in the UUO kidney, including α SMA, Collagen-1 at the mRNA and/or protein levels and Klotho treatment alleviated these changes. The study demonstrated that ADC determined by MRI reflects cell density in renal parenchyma, which correlates the degree of fibrosis and the level of mesenchymal marker expression

The study demonstrated that DWI could evaluate the therapeutic response on the progression of the renal fibrosis. We postulate that DWI-derived ADC can be a validated noninvasive biomarker for the progression or regression of renal fibrosis and it has the potential to reduce the need for renal biopsies during the treatment of patients with CKD.

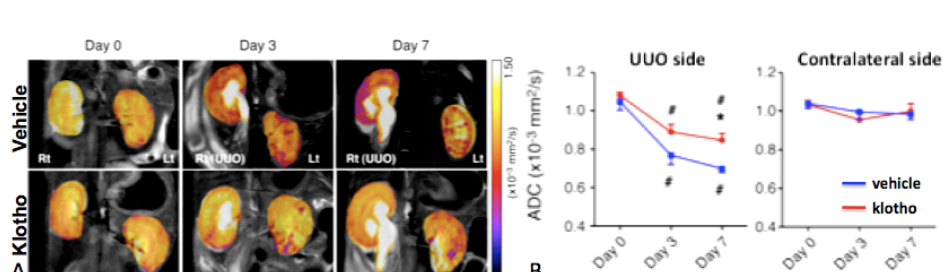


Fig. 1. (A) Typical ADC maps from mice before (day0) and after UUO (day3 and day7) administered with vehicle or Klotho (0.02 mg/kg). (B) Changes in the average ADC values. Data indicate means \pm SEM. *P < 0.05 vs vehicle-treated mice at the same time points by two-tailed t test. #P < 0.05 vs mice at Day 0 by two-tailed t test.

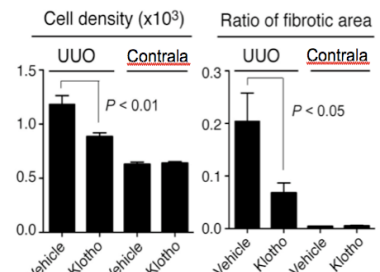


Fig. 2. Quantification of cell density and fibrotic area in UUO and contralateral (contra) sides at day7 after UUO. Data indicate means \pm SEM. P < 0.05 vs vehicle-treated mice by two-tailed t test.

Reference: 1. H.C. Thoeny et al. *Radiology* 255:667-668 (2010). 2. O. Togao et al. *Radiology* 255:772-780 (2010). 3. M. Kuro-o, *Nature* 390:45-51 (1997)