Evaluating Endothelial Damage in Acute Kidney Injury with Perfluorocarbon (PFC) Nanoparticles (NP) and 19F MRI

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Introduction: Endothelial damage is a key pathological feature of acute kidney injury (AKI). AKI causes reduced blood flow, increased endothelial permeability, and abnormal intrarenal oxygenation that are all influenced by various inflammatory mediators and the complex interaction between tubular function and renal microcirculation [1]. Although medical imaging of intrarenal perfusion and its response to therapy in KI patients could be useful for judging the extent of damage and for following therapy, in many cases the use of contrast agents is restricted by the renal toxicity associating with administrated imaging contrast agents. We propose that perfluorocarbon (PFC) based nanoparticles (NP) is a promising and nontoxic candidate for MRI-based molecular imaging of renal damage and perfusion [2]. In fact, the detected ^{19}F signal intensity directly reflects local blood volume, and the ^{19}F longitudinal relaxation time (T1) is inversely proportional to local blood oxygen content (PO_2) [3]. In this study, we sought to investigate renal vascular damage using proposed ^{19}F functional MRI and ^{1}H blood oxygenation dependant (BOLD) MRI [4] in a mouse model of warm ischemia/reperfusion kidney injury.

Method: Male C57BL/6 mice (N=10) were anesthetized with ketamine/xylazine and underwent laparotomy with unilateral renal ischemia by ligating both the left renal artery and vein. Kidney ischemia was maintained for 60 minutes and then ligature was released to resume perfusion. At 24 hours post-injury, mice were anesthetized for ^{1}H and ^{19}F MRI after intravenous injection of 40% v/v Alex Fluor 594 labeled perfluoro-15crown-5-ether (CE) emulsion (5ml/kg). All the MR experiments were carried out on a Varian 11.7 T small animal scanner. A custom built actively-decoupled saddle coil and curved surface coil were used for RF transmission and reception respectively. Both coils were tuned to ^{19}F frequency to achieve maximal SNR for imaging of PFC NP since ^{1}H signal can be effectively detected in this setup. ^{1}H T1-weighted gradient echo imaging was first performed to locate the short axis of both kidneys. To estimate ^{19}F T1, two ^{19}F images were acquired at the identical location with respiration-gated fast spin echo sequences with TR = 2.4 s and TR = 0.4 s, respectively. Other ^{19}F imaging parameters are: number of average = 32, TE = 8 ms, number of echoes = 4, in-plane resolution = 0.39 mm×0.39 mm, and slice thickness = 2 mm. The ratio between two ^{19}F images with different TRs was used for estimating ^{19}F T1 pixel by pixel. After ^{19}F imaging, a ^{1}H multi-echo gradient echo sequence was used to generate ^{1}H T2* map using BOLD MRI. The imaging parameters are: TR = 100 ms, TE = 1.7 ms, number of echoes = 8, flip angle = 10°, slice thickness = 2 mm, number of average = 8, and resolution = 0.78 mm×0.78 mm. Three regions of interest (ROI), i. e. cortex, corticomedullary junction (C-M junction), and medulla were manually determined based on T1-weighted ^{1}H image and ^{19}F image. Throughout the imaging, mice were breathing 100% oxygen through a nose cone. After MRI, mice were perfused with FITC-Lectin to stain endothelial cells. Dissected kidneys were flash-frozen and cryto-sectioned for fluorescence microscopy of the density of perfused renal blood vessels and PFC NP distribution.

Result: In healthy kidneys, ^{19}F intensity images showed that renal blood volume decreases from cortex to C-M junction and increases again in the medulla (Fig. 1b&d). In addition, ^{19}F T1 in medulla (2.82±0.58 s) was longer than that in C-M junction (1.78±0.2 s) and cortex (1.56±0.22 s) (Fig. 1c&e), reflecting lower medulla PO_2. Renal I/R injury induced changes in regional blood volume, vascular leakage and PO_2 was assessed by ^{19}F MRI and BOLD MRI (Fig. 2). Compared to the contra-lateral control kidneys, I/R injured kidneys exhibited decreased ^{19}F intensity but unchanged ^{19}F T1, respectively reflecting reduced blood volume but unchanged PO_2, in C-M junction (Fig. 2). In contrast, BOLD MRI showed increased T2* in C-M junction indicating decreased PO_2 under the assumption of unchanged blood flow. Histological analysis showed the density of perfused blood vessels in C-M junction was reduced. Finally, the inner medulla of I/R injured kidneys exhibited higher ^{19}F signal (0.8±0.19) than that of control (0.45±0.06), agreeing with histologically detected accumulation of PFC NP in the extra-vascular space.

Discussion and Conclusion: We have demonstrated a new ^{19}F MRI approach using circulating PFC NP to quantify renal perfusion and vascular damage after ischemic injury. The measured regional intrarenal blood volume and oxygenation is consistent with previous observations [4, 5]. In I/R injured kidney, ^{19}F MRI of PFC NP delineates reduced blood volume in C-M junction and vascular leakage in medulla as a consequence of endothelial damage. Although BOLD MRI shows enhanced T2* values at C-M junction of injured kidneys, it does not necessarily reflect hyperoxia but may also sense reduced blood flow. Thus, the proposed novel ^{19}F MRI approach combined with BOLD MRI could provide a powerful tool to assess renal vascular damage in AKI.

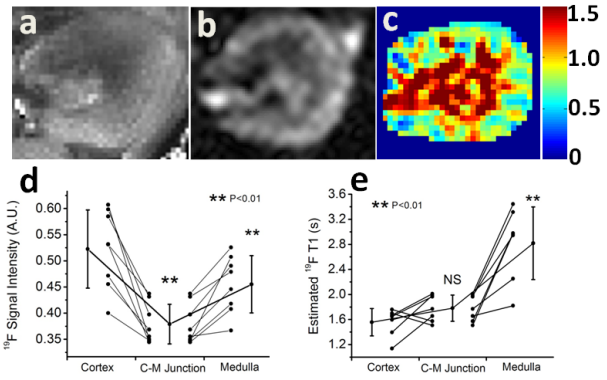

Figure 1. MRI of healthy kidneys. (a) T1-weighted ^{1}H image showed the kidney anatomy. (b) ^{19}F intensity map of PFC NP showed regional blood volume. (c) ^{19}F T1 map showed intrarenal oxygenation (color bar unit: s). (d-e) Quantified of ^{19}F signal intensity and T1 at cortex, cortex-medulla (C-M) junction, and medulla.

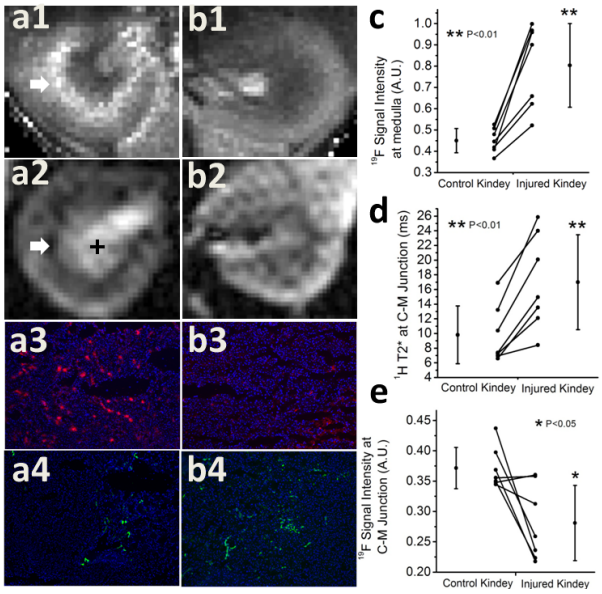

Figure 2. (a1-4) Representative MR and fluorescence images of injured kidneys: 1) BOLD T2* map; 2) ^{19}F intensity map; 3) PFC NP fluorescence image at medulla; and 4) FITC-Lectin fluorescence image at C-M junction. White arrows point at altered ^{1}H T2* and ^{19}F intensity at C-M junction and the black cross indicates enhanced ^{19}F signal intensity at medulla of injured kidney. Blue=DAPI, Red=PFC NP, Green=FITC-Lectin. (b1-4) Corresponding representative images of control kidneys as described in (a1-4). (c-e) Quantification of ^{19}F intensity at medulla, ^{1}H T2* at C-M junction and ^{19}F signal intensity at C-M junction of comparing injured and control kidneys.

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