Assessment of Experimental Acute Kidney Injury by Fast Interleaved Monitoring of T_2* and T_2

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Introduction: Acute kidney injury (AKI) is commonly caused by renal hypoperfusion or temporary interruption of blood flow [1-3]. This ischemia/reperfusion (I/R) injury is characterized by a shift in the fragile balance of local tissue oxygen supply and demand [4-5]. Despite substantial progress in the field of AKI there is an unmet need to better understand the mechanisms operative during the initial phase of I/R injury in AKI. Non-invasive *in vivo* parametric magnetic resonance imaging (MRI) holds the promise to elucidate spatio-temporal pathophysiological changes in the kidney by monitoring the MR relaxation parameters T_2* and T_2, which are known to be sensitive to blood oxygenation. We sought to establish the feasibility of continuous and high temporal resolution parametric MRI for *in-vivo* monitoring and characterization of I/R induced AKI in rats.

Materials and Methods: MRI protocols for parametric mapping of T_2* and T_2 were tailored for rapid measurements in the rat kidney at a 9.4 Tesla (Bruker Biospec, Ettlingen, Germany). Changes in renal T_2*, T_2 were explored during different inspiratory gas composition (hypoxia, hyperoxia) and during renal I/R. ***MR Imaging:*** A four-element RX surface coil array was combined with a 72mm diameter TX volume coil. T_2 mapping used an MSME protocol with TR = 550ms, TE = 10-70ms (7 values), TA = 1:40min. T_2* mapping used an MGE protocol with TR = 50ms, TE = 2.85-28.5ms (10 values), FA = 22°, TA = 1:20min. Renal T_2/T_2* were monitored in an interleaved manner for a coronal oblique slice (FOV = 38.2x50.3mm^2, matrix = 169x215, in plane resolution = (230x230)µm^2, slice thickness = 1.4-1.5mm). ***Animal Model:*** Experiments were performed on two groups of six male 2-4 months old rats, weighing between 250-350g. Animals were anesthetized using urethane (Sigma-Aldrich, Germany; 20%; 6 ml/kg i.p.). Body temperature was maintained at 37°C. ***Hypoxia/Hyperoxia Experiments:*** The suitability of T_2*/T_2 mapping for the detection of renal blood oxygenation changes was first demonstrated by examining the MRI protocol's sensitivity to externally controlled variations of blood oxygenation. This was done by exposing one group of animals to brief periods of hypoxia (10% oxygen, 90% nitrogen) and hyperoxia (100% oxygen) lasting 8 minutes. During baseline (~15min) and post stimulus recovery (~15min) room air was provided (normoxia). ***Ischemia/Reperfusion Experiments:*** In the second group of animals a remote controlled hydraulic occluder was placed around left renal artery and vein after right uninephrectomy to allow for induction of renal ischemia during continuous imaging. Following a baseline of five measurements, fast T_2*/T_2 mapping was performed throughout 45min of warm ischemia until 100min after reperfusion with a temporal resolution of 3min. Interruption of renal blood flow was confirmed by TOF MRA before and after induction of ischemia. ***Segmentation Model Based Analysis:*** For examination of T_2*/T_2 changes a standardized kidney segmentation model was developed. The model includes nine ROIs strictly following morphological kidney features, i.e. three in each of the distinct renal layers: cortex, outer medulla, and inner medulla. The dimensions of the rat kidney layers were measured in freshly harvested kidneys as well as formalin-fixed kidneys (n=16). To account for the inter-individual variability in kidney length and width, a rectangular frame that tightly encloses the kidney in the coronal view was used as a reference to define the relative positions of the ROIs. Size and positions of the ROIs were chosen such that they are far away from the borders between the kidney layers to avoid any 'contamination' from the neighboring layers and allow for inter-individual variations in morphology. Implementation of this model in a semi-automated analysis program developed in ImageJ (NIH, USA) limited user interaction to the placement of the rectangular reference frame around the kidney.

Results: T_2* and T_2 in all kidney layers showed great sensitivity to changes in inhaled gas composition to 10% O_2 or 100% O_2 (Fig. 2). During ischemia/reperfusion substantial alterations in T_2* and T_2 were observed (Fig. 1,2,3). Cortical T_2* returned to baseline after restoration of renal blood flow (Fig. 2,3). Cortical T_2 increased by 25% compared to baseline after reperfusion (Fig. 2). In contrast, in the outer medulla T_2*/T_2 was approx. 70%/35% below baseline after ischemia (Fig. 3), which correlated with the region of most severe morphologic damage.

Discussion and Conclusions: Our study demonstrates for the first time that continuous *in vivo* parametric MRI monitoring of renal I/R is feasible. This approach enabled the detailed assessment of *in vivo* changes in T_2* and T_2 for all kidney regions during ischemia and early reperfusion. Observations in the early reperfusion phase promise to offer new insights into the pathogenesis of I/R AKI and might help to identify the timeline of key events responsible for development of cellular damage. The method of parametric MR monitoring may also be a useful investigational tool for other models of AKI.

References: [1] de Mendonca, Intensive Care Med 2000, [2] Chertow, J Am Soc Nephrol 2005 [3] Hoste, Crit Care Med 2008, [4] Nash, Am J Kidney Dis 2002, [5] Schrier, J Clin Invest 2004.

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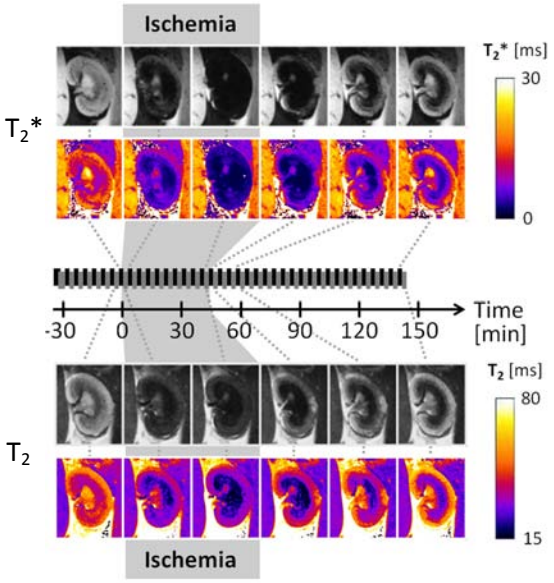

Figure 1: Ischemia reperfusion experiments: T_2*- and T_2-weighted MR images (gray scale) together with color-coded T_2* and T_2 parameter maps for 6 of 55 time points. The parameter maps demonstrate immediate changes in T_2*/T_2 after start and end of ischemia. Ischemia led to a significant T_2* and T_2 decrease.

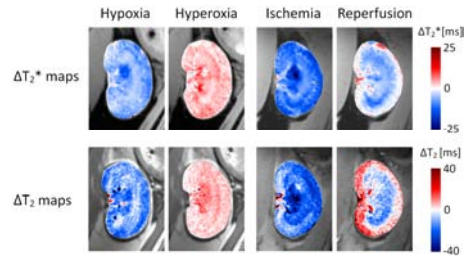

Figure 2: Change of renal T_2* and T_2 during hypoxia, hyperoxia, ischemia and reperfusion. Shown are T_2* and T_2 difference maps of the kidney (color-coded, overlay on anatomical MR image) between the last time point in each experiment phase and baseline.

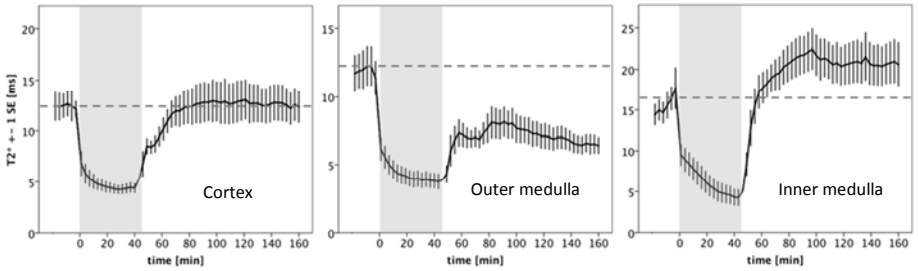

Figure 3: Ischemia reperfusion results derived from the standardized segmentation model of the rat kidney: Plots of T_2* (mean ±SEM averaged over six animals). Ischemia (shaded in gray) led to an immediate and significant T_2* decrease in all kidney ROIs. At the end of the reperfusion period T_2* was close to baseline (dashed line) in the cortex, below baseline in the outer medulla, and above baseline in the inner medulla. The three ROIs within each kidney layer showed very similar trends.