Parametric Mapping of Renal T2* Demonstrates Beneficial Effect of Epoxyeicosatrienoic Acid for Preventing Acute Kidney Injury

Andreas Pohlmann[1], Jan Hentschel[1], Uwe Hoff[2], Gordana Bubalo[2], Mandy Fechner[2], Maximilian Blum[3], Ye Zhu[2], Karen Arakelyan[1,4], Erdmann Seeliger[4], Bert Flemming[4], Dennis Gurgen[2], Wolfgang Schneider[3], Michael Rothe[5], Vijaya L Manthati[6], John R Falck[6], Wolf-Hagen Schunck[3], Duska Dragun[2], and Thoralf Niendorf[1]

[1]Berlin Ultrahigh Field Facility, Max Delbrueck Center for Moleculare Medicine, Berlin, Germany, [2]Nephrology and Intensive Care Medicine, Campus Virchow-Klinikum and Center for Cardiovascular Research, Charite-Universitaetsmedizin Berlin, Berlin, Germany, [3]Experimental and Clinical Research Center, Charite Medical Faculty and the Max Delbrueck Center for Molecular Medicine, Berlin, Germany, [4]Institute of Physiology and Center for Cardiovascular Research, Charité Medical Faculty, Berlin, Germany, [5]Lipidomix GmbH, Berlin, Germany, [6]Biochemistry Department, UT Southwestern, Dallas, TX, United States

Target audience: MR scientists, clinicians, clinical scientists and basic MR researchers with an interest in acute kidney injury.

Introduction and Purpose: Two million per year is the estimated worldwide death toll of AKI,[1] with renal ischemia-reperfusion (I/R) being one of the major causes of acute kidney injury (AKI).[2] Current therapeutic options for treating AKI are disappointing and hence the need for establishing druggable targets for ameliorating renal injury. Imbalances in cytochrome P450 (CYP)-dependent eicosanoid formation may play a central role in AKI. Pharmacologic inhibition of 20-hydroxyeicosatetraenoic acid (20-HETE) action ameliorated I/R-induced AKI in rats.[3] Here we tested the hypothesis that early pharmacologic enhancement of epoxyeicosatrienoic acid (EET) actions may counteract the detrimental effects of 20-HETE and prevent the initiation of AKI. Renal I/R injury is characterized by mismatch of local tissue oxygen supply and local cellular energy demand and results in renal injury, which is clinically detected as compromised glomerular filtration rate.[4] This imbalance between oxygen demand and supply is considered to be the initiating step in the pathophysiologic cascade of events.[5] In this study we investigated whether administration of an EET agonist improves cortical and medullary re-oxygenation as monitored by *in vivo* parametric MRI during the initial 2 h reperfusion phase.

Methods: ***Animal model:*** 17 male Lewis rats (age 2-3 months, 250–300g) underwent experimental ischemia/reperfusion (I/R) injury inside a 9.4T MR system (Bruker Biospin, Ettlingen, Germany).[6] The rats were anesthetized using urethane (20% in water, 6 ml/kg body mass) and kept at a constant core body temperature of 37°C during surgery and MRI. After right nephrectomy an aortic catheter was placed with its tip directly at the left renal branch for intrarenal administration of an EET agonist or vehicle. The rats were transferred into the MR scanner and T_2* was continuously monitored with a temporal resolution of ~3 min. Ischemia was induced by closing a remotely controlled hydraulic occluder around the renal artery and vein for 45 minutes,[6] followed by a reperfusion phase of ~100 minutes. Interruption of renal blood flow was confirmed by time-of-flight MR angiography of the kidney. ***MR imaging:*** Experiments were carried with a birdcage RF resonator (TX) in conjunction with a four channel RF coil array (RX; Bruker Biospin, Germany) customized for rats. T_2* mapping used a respiratory gated multi gradient echo (MGE) sequence (TR = 50 ms, number of echoes = 10, first TE = 1.43 ms, echo spacing = 2.14 ms, averages = 4) with a total acquisition time of approx. 1 min 20 s. A coronal oblique image slice was acquired with a spatial in plane resolution of (226x445) μm^2 (FOV = (38.2x50.3) mm^2, matrix size = 169x113 zero-filled to 169x215) and a slice thickness of 1.4 mm. ***Statistics:*** Results were expressed as mean ±SEM. Cortical and outer medullary T_2* derived from six regions-of-interest [6] per kidney were statistically analyzed using SPSS (IBM, Germany). Following Kolmogorov-Smirnov test to confirm normal distribution and Levene's test to conform equality of variances, two-tailed t-tests were performed for differences to baseline and differences between the EET agonist group and vehicle group (mean of last 5 timepoints during reperfusion, $p<0.01$, corrected for multiple comparisons.

Results: After ischemia T_2* of the outer medulla remained significantly (ΔT_2* = 4.7 ms) below baseline level during the 2 hours post reperfusion period (Fig.1A). In contrast, cortical T_2* exceeded baseline levels in the initial reperfusion phase. Outer medullary T_2* levels were significantly higher after pretreatment with the EET agonist versus vehicle, but were not restored to pre-ischemia level. Cortical T_2* levels remained significantly elevated in the EET agonist group, whereas in vehicle treated animals T_2* slowly returned to baseline. After treatment with the EET agonist T_2* ratio maps (T_2*$_{end\text{-}reperf}$ / T_2*$_{baseline}$) illustrated in Fig.1B indicate markedly improved re-oxygenation in the cortex and outer medulla 2 hours post reperfusion versus vehicle control (Fig.1B).

Discussion and Conclusion: This study shows that pretreating the kidney with an EET agonist significantly alleviates I/R-induced renal medullary hypoxia in an experimental model of AKI. Sustained medullary vasoconstriction in the reperfusion phase delays the recovery of medullary perfusion and oxygenation and thus contributes to I/R-induced renal injury.[3] Monitoring T_2* time courses, we found that the EET agonist improved cortical and outer medullary re-oxygenation during the early reperfusion phase. EETs mediate vasodilation of renal arterioles by stimulating calcium-activated potassium channels in vascular smooth muscle cells.[7] Moreover, EETs inhibit salt reabsorption in proximal and distal tubules by inhibiting the Na^+/H^+-exchanger and the epithelial Na^+-channel respectively.[8] Thus, the EET agonist might have improved renal re-oxygenation by both increasing microvascular oxygen supply and reducing tubular oxygen consumption. As changes in T_2* are related to the local concentration of deoxyhemoglobin they largely reflect variations in blood oxygenation, but may also by influenced by vasomotion and hematocrit. EET-mediated vasodilation increases the blood volume fraction and hence increases renal deoxyhemoglobin amount per tissue volume (i.e. decreases T_2*) even at unchanged blood oxygenation. The observed EET-induced increase in T_2* might therefore underestimate the actual improvement of blood re-oxygenation. In summary, parametric mapping of renal T_2* demonstrates improved renal re-oxygenation after EET treatment. EET agonists administered in the initiation phase of renal I/R-injury may provide novel therapeutic options for the prevention of ischemic AKI.

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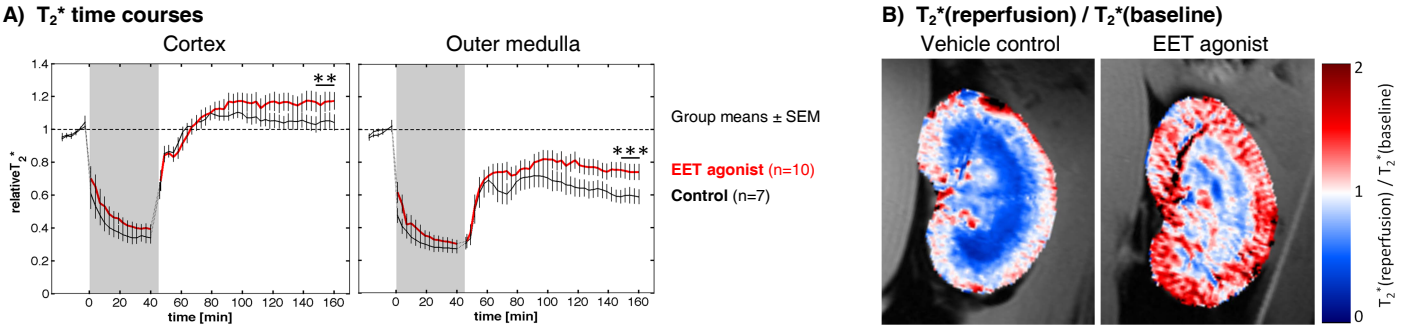

Figure 1: EET agonist improves renal oxygenation measured by high temporal resolution T_2* mapping during the initial ischemia/reperfusion phase. Kinetics of T_2* changes (a) and color-coded T_2* ratio maps (reperfusion/baseline) of two exemplary kidneys superimposed to the anatomical MR image (b) show lower extent of hypoxia and better re-oxygenation after EET agonist treatment. Statistically significant differences (2-tailed t-test) were observed as indicated: ** $p<0.01$ vs vehicle, *** $p<0.001$ vs vehicle.