Detection of evolving acute tubular necrosis with renal ^{23}Na MRI: Studies in rats

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The clinical detection of evolving acute tubular necrosis (ATN) and differentiating it from other causes of renal failure are currently limited. The maintenance of the corticomedullary sodium gradient, an indicator of normal kidney function, is presumably lost early in the course of ATN. Herein, sodium magnetic resonance imaging (^{23}Na MRI) was applied to study the early alteration in renal sodium distribution in rat kidneys 6 h after the induction of ATN. Three-dimensional gradient echo sodium images were recorded at 4.7 T with high spatial resolution. ATN was produced by the administration of radiologic contrast medium, combined with inhibition of nitric oxide and prostaglandin synthesis. The sodium images revealed that the sham-controlled kidney exhibited a linear increase in sodium concentration along the corticomedullary axis of 30 ± 2 mmol/l/mm, resulting in an inner medulla to cortex sodium ratio of 4.3 ± 0.3 ($n = 5$). In the ATN kidney, however, the cortico-outer medullary sodium gradient was reduced by 21% ($P < 0.01$, $n = 7$) and the inner medulla to cortex sodium ratio was decreased by 40% ($P < 0.001$, $n = 7$). Small, though significant, increments in plasma creatinine at this time inversely correlated with the decline in the corticomedullary sodium gradient. Histological findings demonstrated outer medullary ATN involving 4% of medullary thick ascending limbs. Hence, ^{23}Na MRI non-invasively quantified changes in the corticomedullary sodium gradient in the ATN kidney when morphologic tubular injury was still focal and very limited. MRI detection of corticomedullary sodium gradient abnormalities may serve to identify evolving ATN at its early phases.

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The maintenance of the corticomedullary sodium gradient is a basic renal function, required for urine concentration. This gradient, produced by the countercurrent system, depends on the active reabsorption of sodium by ion pumps in the medullary thick ascending limb (mTAL), the difference in the permeability characters of the descending and the ascending limbs, and the unique paralleled structure of the nephron segments and vasculature in the medulla.[1,2] The outer medulla normally functions at very low oxygen tension, in part due to high oxygen consumption by those ion pumps.[3] This region is, therefore, vulnerable to injury, which plays an important role in the evolution of hypoxic acute tubular necrosis (ATN). Indeed, a declining urinary osmolarity, a hallmark of a disrupted concentrating system, is considered a very early sign of evolving ATN.[4–8] Clinical identification of early-stage ATN and its differentiation from other causes of acute renal failure are currently limited. The diagnosis of ATN is often done at an advanced, established phase, beyond the time-point of potential preventive intervention. Therefore, non-invasive real-time detection of the altered corticomedullary sodium gradient could serve for early detection of evolving ATN.

Sodium magnetic resonance imaging (^{23}Na MRI) provides the unique ability to directly measure and determine the tissue sodium concentration (TSC) non-invasively.[9–15] Early studies showed the ability of ^{23}Na MRI to detect the corticomedullary sodium gradient in exposed kidneys.[16–18] We have recently demonstrated the use of sodium MRI to non-invasively quantify the corticomedullary TSC gradient in the intact rat kidney, and followed its modulation in hydronephrosis.[19] Moreover, changes in outer medullary sodium content were specific and served to identify functional differences during mannitol- and furosemide-induced diuresis.[20] We hypothesized that the early evolution of hypoxic ATN may manifest with the development of altered corticomedullary sodium gradient, detected non-invasively by sodium MRI.

RESULTS

At 6 h after the insult, plasma creatinine rose to 0.59 ± 0.02 mg/dl ($n = 7$) in the ATN group, as compared with sham-injected control rats (0.38 ± 0.03 mg/dl, $P < 0005$,

$n = 5$). Morphologic evaluation revealed limited tubular injury, involving $4.2 \pm 1.9\%$ of mTALs in the mid-inner strip (Figure 1), particularly in the mid-interbundle zone, most remote from the vasa recta.

The sodium distribution in the kidneys of control rats was similar to that observed previously in normal rat kidneys ($n = 20$).[19] The images revealed a gradual increase of the sodium signal intensity from the cortex along the corticomedullary axis, reaching its highest value in the inner medulla (Figure 2a). Conversion of the units from signal intensity to concentration yielded a TSC gradient along the corticomedullary axis of 30 ± 2 mmol/l/mm.

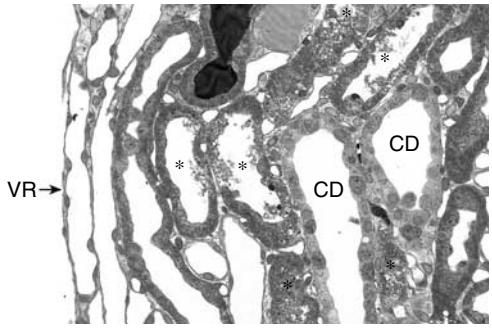

Figure 1 | Histopathology of an outer medullary section of an ATN kidney. The kidneys were fixed with 1.25% glutaraldehyde solution 6 h after the insult, and stained with 1% methylene blue. Focal mTAL injury (*) can be seen at high magnification ($\times$ 400) adjacent to collecting ducts (CD) and away from the vasa recta (VR). Tubular injury was basically limited to 4% of mTALs in the mid-inner stripe.

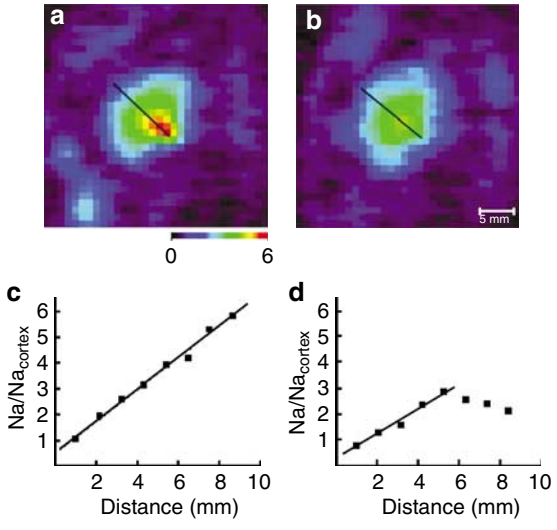

Figure 2 | Renal sodium distribution under conditions of ATN. Sodium images of rat kidneys recorded 6 h after (**a**) sham injection and (**b**) composite injections of indomethacin, N^{ω}-nitro-L-arginine methyl ester, and iothalamate as described in Materials and Methods. Images were recorded using a 3D gradient echo sequence with a TE/TR of 1.7/60 ms, spatial resolution of $0.94 \times 0.94 \times 5\ mm^3$, and 20 min scanning time. (**c**, **d**) The sodium content, relative to that in the cortex, along the corticomedullary axis (marked with a black line on the images) versus the distance from the cortex outer edge.

Renal sodium images, recorded 6 h after inducing ATN, were clearly different from those of the sham-injected rats (Figure 2b). The strongest deviation was observed in the inner medulla, where the TSC declined by $42 \pm 6\%$ compared to that in the sham-controlled kidney. Consequently, the inner medulla to cortex sodium ratio was reduced from 4.3 ± 0.3 in sham control to 2.6 ± 0.2 ($P < 0.001$) in ATN kidneys. Analysis at pixel resolution revealed a marked change in the profile of TSC along the corticomedullary axis (Figure 2c and d). In contrast to the linear profile observed in the control kidneys throughout the corticomedullary axis, its contour in the ATN kidneys contained a positive linear slope (average linear correlation factor $R = 0.96 \pm 0.04$) from the cortex to the outer medulla, and a negative slope from the outer to the inner medulla. Furthermore, the positive slope decreased significantly ($P < 0.01$) by $21 \pm 2\%$ to 23.7 ± 1.5 mmol/l/mm in reference to that in the sham control kidney (30 ± 1 mmol/l/mm). Interestingly, even at this early stage of ATN, the reduction of cortico-outer medullary sodium gradient correlated with the small increments in plasma creatinine (Figure 3; $R = 0.84$).

DISCUSSION

Early detection of evolving ATN and its differentiation from pre-renal causes of kidney dysfunction have major potential implications regarding the patient's management. However, clinically applicable diagnostic tools of ATN are currently insufficient. Functional indicators of urinary concentration and urinary sodium are useful diagnostic tools, but require freshly generated urine, and may be influenced to a great extent by therapeutic interventions.

A loss of urinary concentrating capacity is an early indicator of ATN, reflecting altered countercurrent system and urine concentration capacity.[4–8] Non-invasive detection of the corticomedullary sodium gradient may, therefore, serve as an early real-time indicator of evolving ATN. Herein we show that the renal sodium MR images revealed a marked

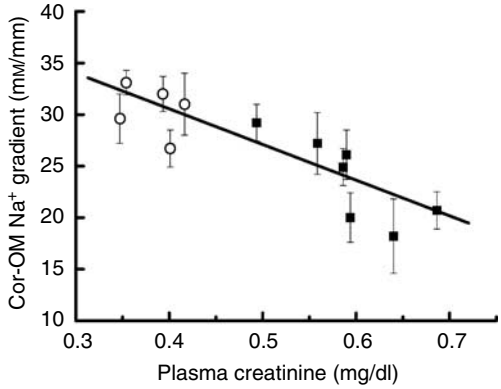

Figure 3 | Correlation between the cortico-outer medullary (Cor-OM) sodium gradient and plasma creatinine. The gradients of sham-controlled (○) and ATN (■) kidneys were calculated from the sodium images, using pixel-by-pixel analysis, as shown in Figure 2. The plasma creatinine was measured immediately after the MRI experiment.

change in the corticomedullary sodium signal intensity profile during the early evolution of ATN. In general, the sodium signal intensity is determined by the TSC, as well as by the sodium longitude and transverse relaxation rates. In living tissue, the relaxation mechanisms of sodium are determined by the interaction of the sodium ions with macromolecules.[21] These relaxation rates were measured previously in the intact rat kidney and found to be similar in the cortex and the medulla.[19] Moreover, extreme changes in tissue composition that resulted from diuresis did not affect these rates.[20] Since the observed histological damage in the ATN kidneys was minimal, it is reasonable to assume that the sodium relaxation rates remained similar to those in the normal kidney. Therefore, the observed changes in the sodium signal intensity in the ATN kidneys with respect to the sham control kidneys reflected the corresponding changes in renal TSC.

The sodium images clearly demonstrated the inability of the ATN kidneys to maintain the corticomedullary sodium gradient; the slope of the cortico-outer medullary gradient declined and the inner medulla to cortex sodium ratio decreased. Declining corticomedullary sodium gradient conceivably reflects a functional derangement of the countercurrent system, related to tubular damage or malfunction, and perhaps to evolving regional endothelial dysfunction. The fact that water deprivation did not affect the sodium gradient[19] illustrates the potential of this technique to distinguish between pre-renal azothemia and ATN.

Hypoxic tubular necrosis, in models of distal tubular injury, is very limited initially, but gradually develops over 24 h after insult.[22] Indeed, only 4% of mTALs in the mid-inner stripe were necrotic by 6 h, the time the MRI study was performed. However, apoptotic cells and tubules with mild, potentially reversible features of morphologic hypoxic tubular injury were previously shown to transiently coexist at that stage,[22,23] and presumably contribute to the impaired regional concentrating capacity. A widespread expression of hypoxia-inducible factors both in tubular segments and in endothelial cells in the renal medulla illustrates the extent of regional hypoxic stress produced in this model.[24] Furthermore, the loop diuretic furosemide inhibits the mTAL sodium transporter and consequently abolishes outer medullary hypoxic injury.[25] We have previously shown that indeed the cortico-outer medullary sodium gradient almost disappears in the presence of furosemide,[20] whereas no change in this gradient is observed in mannitol-induced diuresis, where mTAL pumps maintain their function. Thus, a direct coupling exists between tubular transport dysfunction, induced by either hypoxic insults or by transport inhibition, and a reduction in the cortico-outer medullary sodium gradient.

Collectively, our sodium MRI studies indicate that altered cortico-outer medullary sodium gradient selectively represents a malfunctioning countercurrent system, whereas a reduction in the inner medullary sodium is non-specific.[19,20] The observed reduction in the inner medulla sodium in ATN probably reflects regional structural damage, also quite prevalent in this model,[24,23] and in the hydronephrotic kidney.[19] A similar pattern of flattened inner medullary sodium gradient is also seen following diuretics,[20] presumably representing the washout of papillary sodium content.

In summary, we have demonstrated the ability of ^{23}Na MRI to quantify changes in the corticomedullary sodium gradient at early stages of ATN when morphologic tubular injury is still focal and very limited. This noninvasive technique may enable the detection of evolving ATN in the setup of acute renal failure, and to differentiate it from the pre-renal phase, where tubular function is well maintained.

MATERIALS AND METHODS

ATN model

The study was performed on female 2–4-months-old Lewis rats (250–300 g), and was approved and conducted in accordance with the guidelines of the Institutional Committee on Animals of the Weizmann Institute of Science. Medullary hypoxic ATN was induced by the administration of the radiocontrast sodium iothalamate (Angio-Conray 80%, Mallinckrodt, St Louis, MO, USA; 6 ml/kg intraarterial) following the inhibition of prostaglandin synthesis with indomethacin (Sigma Chemical Co., St Louis, MO, USA; 2 mg/kg, intravenous) and of nitric oxide synthesis with N^{ω}-nitro-L-arginine methyl ester (Sigma Chemical Co., St Louis, MO, USA; 1 mg/kg, intravenous), as described previously.[26] Outer medullary hypoxic stress and tubular damage have been documented early after the induction of ATN in similar[24] and comparable experimental setups.[22]

Rats were anesthetized by exposure to 1% isoflurane (Medeva Pharmaceuticals, Inc., Rochester, NY, USA), in an O_2/N_2O (3:7) mixture, applied through a nose cone. In seven rats (ATN group) indomethacin, N^{ω}-nitro-L-arginine methyl ester and sodium iothalamate were sequentially injected at 15-min intervals through the rat-tail veins and artery. Five additional animals injected with vehicles served as sham controls. After 6 h MR images were recorded, as described below. Subsequently, plasma samples were obtained for the determination of creatinine. In seven different rats, the kidneys were perfusion-fixed 6 h after the insult and processed for morphologic assessment, as detailed elsewhere.[22,23,27]

MRI

The study was performed on a 4.7-T Biospec spectrometer (Bruker, Rheinstetten, Germany) using a home-built 3 cm ^{1}H/^{23}Na double-tuned surface coil. The rats were anesthetized by sodium pentobarbital (0.04 mg/g, intraperitoneal), and were placed on the coil in a supine position.

The location of the kidney along the axial axis was chosen by carefully positioning the height of the rat, to avoid dislocation of the center of the kidney in the sodium image due to the small number of axial sampling points ($n = 16$). This was achieved by localizing the exact position of the kidney center using axial 3D ^{1}H gradient echo images acquired at high resolution,[19] and then setting the height of the medulla from the magnet center to be an integer (between 0 to 3) times the slice thickness. After this fine positioning of the rat, an oblique coronal–sagittal field of view (FOV) was assigned for the sodium image so that the angle of the oblique plane was equal to that of the corticomedullary axis. The 3D ^{23}Na gradient echo image was then recorded, using a 90° sine/cosine adiabatic pulse,[28] a echo-time/

repetition-time (TE/TR) of 1.7/60 ms, FOV $12 \times 12 \times 5$–$8\,cm^3$, and a matrix of $128 \times 128 \times 16$ with 10 scans (20 min).

The conversion of the relative signal intensity units to TSC units was based on sodium imaging of a reference saline solution, taking into account the sodium relaxation rates in the reference solution and in the rat kidney, measured previously,[19] and correcting for coil sensitivity.[20] In order to define the exact boundaries of the kidney regions (cortex, outer and inner medulla) in the sodium image, an oblique 3D ^1H MR image was also acquired with the same FOV and spatial resolution as that of the sodium image.[19]

Statistical analysis

Results are presented as the mean $\pm$ s.e.m. Two-tailed unpaired Student's *t*-test was used for the comparison of control and ATN kidney and linear regression fitting was applied to assess MRI and functional correlations.

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