Detection of acute kidney injury using hyperpolarized [1,4-13C2]fumarate

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Acute kidney injury (AKI), is a common and clinically important problem, affecting between 5 and 10% of all hospitalised patients, and 30 to 40% of those admitted to a critical care setting. Acute tubular necrosis (ATN), characterised histologically by flattening of tubular epithelium, tubular dilatation, and loss of tubular cell nuclei, and functionally by loss of electrolyte balance, leading to high urinary sodium, is the most common cause of AKI (1). The main important differential diagnoses in patients presenting with AKI is rapidly progressive glomerulonephritis (GN), which can occur secondary to autoimmune diseases. Treatment of the two conditions differs. Currently, however, there is no non-invasive test which differentiates ATN and GN, as the standard mode of imaging (ultrasound examination) demonstrates normal-sized, unobstructed kidneys in both cases.

Appearance of [1,4-13C2]malate signal following injection of hyperpolarized [1,4-13C2]fumarate has recently been suggested as a novel marker of necrosis (2-4). In this study, we have investigated whether hyperpolarized [1,4-13C2]fumarate could be a useful tool in the diagnosis of ATN, induced by folic acid administration (5), and whether this technique would allow ATN to be distinguished from acute inflammatory glomerulonephritis (GN).

Methods
Folic acid was dissolved in 150 mM sodium bicarbonate and administered intra-peritoneally at a dose of 250 mg/kg to C57BL/6 mice (n=26). Animals were imaged before folate injection and 10 h, 18 h, 26 h and 48 h post-injection (up to 3 time-point/animal). For GN, we used a model of lupus nephritis, the New Zealand Mixed (NZM)2410 lupus-prone congenic strain (n=6). For MR experiments, animals were anesthetized with intra-peritoneal sodium avertin (0.2 ml/kg body weight) and maintained using 1.1 ± 0.7 % and remained at this concentration during the experiment. For GN, we used a model of lupus nephritis, the New Zealand Mixed (NZM)2410 lupus-prone congenic strain (n=6). For MR experiments, animals were anesthetized with intra-peritoneal injections of Hypnorm (VetaPharma) and Hynovel(Roche)/dextrose-saline (4%/0.18%) in a 5:4:3:1 ratio (10 ml/kg body weight). [1,4-13C2]fumarate was polarized as described previously (2). All experiments were performed at 9.4 T using a 24-mm surface coil placed over the kidneys. 13C spectroscopy and spectroscopic imaging were started 20 s after i.v. injection of 200 µl of fumarate. A single 13C spectrum was first collected from the whole slice using a 600 µs sinc pulse with a nominal flip angle of 5° (2 ms, spectral width 8000 Hz) and this was followed immediately by CSI (TR 20 ms, spectral width 8000 Hz, field of view 40x40 mm², data matrix 16x16). Blood samples were analyzed for serum urea levels after each time point. Tubulointerstitial damage was assessed by scoring three parameters: tubular necrosis, tubular dilatation, and cast formation. Scores were given as follows: involvement of 0 to 25% of tubules within each cortical or medullary high-powered field of view = 1; 25 to 50% = 2; 50 to 75% = 3; 75 to 100% = 4. All values are given as mean±SD.

Results
Only low levels of malate were detected in ATN animals before folate treatment; the malate/fumarate signal intensity ratio was 0.6 ± 0.9 %. In contrast, a prominent malate signal, that was localized to the kidneys, was observed following injection of hyperpolarized [1,4-13C2]fumarate. This signal reached a maximum intensity at 18 h post-treatment, at which time point we observed the most severe histological changes. The malate signal was no longer visible at 48 h post-treatment. In contrast, a prominent malate signal, that was localized to the kidneys, was observed following injection of hyperpolarized [1,4-13C2]fumarate. This signal reached a maximum intensity at 18 h post-treatment, at which time point we observed the most severe histological changes. The malate signal was no longer visible at 48 h post-treatment. The malate/fumarate ratio decreased to 0.4 ± 0.6 % at 48 h post-treatment. The malate/fumarate ratio was not significantly increased in NZM mice compared to control C57BL/6 animals.

Discussion
Increased malate signals were observed 18 hours after folate treatment, at which time point there was histological evidence of tubular necrosis. A significant increase in the malate/fumarate ratio was observed in mice with lupus nephritis. Thus, this technique could allow distinction of ATN from glomerular inflammation in the context of renal impairment. Interestingly, the malate/fumarate ratios had returned to near normal levels when the clinical and histological features of the disease were most severe. This suggests that when there is significant tubular necrosis, there may be loss of fumarase from damaged tubular cells which leads to loss of the malate signal at later time-points. Alternatively, the delivery of fumarate may be altered at the later time points.

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References