NMR-Based Metabolic Profiling of Three Mouse Models of Acute Kidney Injury

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NMR-based metabolic profiling has been used to investigate three mouse models of acute kidney injury (AKI): ischemia reperfusion injury (IRI), hypoxia, and sepsis.

In the IRI-AKI study, 17 mice were subjected to IRI by clamping the renal artery and vein to block blood flow to the kidneys for 30 minutes then the clamp was removed to allow reperfusion. Urine sample were collected 24 hours after IRI then mice were sacrificed and blood and kidneys were harvested. Histological analyses indicated distended Bowman's glomerular spaces and proximal and distal tubules. Increased filtrate volume in nephrons was caused by reduced water reabsorption by severely damaged proximal tubule brush borders and blocked flow of filtrate into collecting tubules by mucoprotein casts in distal tubules. Immunohistochemistry revealed protein AKI biomarkers in proximal tubules and glomeruli but not in distal tubules. NMR spectroscopy revealed several metabolites that increased such as valine alanine and lactate. Other metabolites such as trigonelline, succinate, 2-oxoisocaproate, and 1- methyl-nicotinamide decreased or were absent in urine following IRI due to altered kidney function or metabolism. Urinary glucose increased due to reduced reabsorption by damaged proximal tubule brush borders. Scanning electron microscopy revealed flattening of podocytes and pedicals surrounding glomerular capillaries and transmission electron microscopy (TEM) revealed effacement of podocyte pedicals, both consistent with increased hydrostatic pressure in nephrons following IRI-AKI. TEM revealed shortened proximal tubule microvilli in IRI kidneys with diminished lamina propia. TEM showed dramatic loss of mitochondria in distal tubule epithelia of IRI kidneys and emergence of multivesicular bodies of endosomes indicating ongoing cellular death. Collectively, the data defined ultrastructural changes to nephrons and altered kidney metabolism associated with IRI-AKI.

In the hypoxia-induced AKI study, urine metabolic profiles of 48 Swiss Webster mice were assessed by NMR for 7 days following 72 hours exposure to a hypoxic 6.5% oxygen environment. Histological analyses indicated a lack of gross nephron structural changes in the aftermath of hypoxia. Immunohistochemical (IHC) analyses, however, indicated elevated expression of protein injury biomarkers in distal and proximal tubules but not glomeruli. KIM-1 levels peaked in distal tubules at 72 h and were still increasing in proximal tubules at 7 d post hypoxia, whereas cystatin C levels were elevated at 24 h but decreased thereafter, and were elevated and still increasing in proximal tubules at 7 d post hypoxia. NGAL levels were modestly elevated from 24 h to 7 d post hypoxia. NMR-based metabolic profiling revealed that urine metabolites involved in energy metabolism and associated biosynthetic pathways were initially decreased at 24 h post hypoxia, consistent with metabolic suppression as a mechanism for cell survival, but were significantly elevated at 48 h and 72 h post hypoxia, indicating a burst in organism metabolism associated with reactivation of cellular energetics during recovery after cessation of hypoxia and return to a normoxic environment. The IHC results indicated that kidney injury persists long after plasma and urine biomarkers of hypoxia return to normal values.

In the sepsis-induced AKI study, 12 mice were subjected to a cecal ligation and puncture procedure (CLP). Urine, blood and tissue samples were collected 24 hours after the CLP. NMR data collection for this study is ongoing and will be presented at the meeting.