

# DNA REPLICATION AND PROTEIN TURN-OVER IN POST-ISCHEMIC KIDNEY REPAIR STUDIED WITH MULTI-ISOTOPE IMAGING MASS SPECTROMETRY (MIMS)

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Ischemic preconditioning may lead to important insight into how the kidney uses endogenous mechanisms to protect itself against injury (Bonventre, 2002). We used the established mouse model (J.V. Bonventre) which minimizes the number of residual non-specific responses to the initial intervention at the time when we study the mechanisms of protection. Experiments demonstrate that 30 minutes of bilateral renal ischemia, resulting in significant impairment of renal function as measured by an increase of blood urea nitrogen and creatinine, leads to protection of the mouse kidney against a subsequent ischemic insult 8 or 15 days later, even when the second ischemic period is extended to 35 minutes. Graded levels of time of initial ischemia resulted in graded levels of protection 8 days later. This protection is unrelated to systemic effects of transient uremia since unilateral ischemia is also associated with protection under conditions where there is very little increase in systemic BUN or creatinine. Bromodeoxyuridine (BrdU) and <sup>15</sup>N-L-leucine were administered intra-peritoneally to animals subsequent to the first ischemia to characterize the different responses in cell proliferation in preconditioned kidneys exposed to ischemia on day 8 after the initial surgery. MIMS images were recorded from thin sections of epon embedded kidneys. The samples were analyzed 3 times. A first rapid analysis at low resolution was used to obtain a complete overview of the renal section and identify cells that may have replicated DNA (recording in parallel mass <sup>12</sup>C<sup>14</sup>N, <sup>31</sup>P and <sup>81</sup>Br; field of 100μm x 100μm; 256 x 256 pixels; dwell time: 1ms/pixel). Areas where DNA replication was suspected were reanalyzed two other times at higher resolution, fields of (16μm)<sup>2</sup> to (20μm)<sup>2</sup>. The images were acquired in parallel at mass <sup>12</sup>C<sup>14</sup>N to obtain the morphological details, at mass <sup>31</sup>P to view the cell nuclei and at mass <sup>81</sup>Br to identify the nuclei undergoing DNA replication shown by BrdU incorporation. A second analysis was then performed in the same areas quantitatively imaging in parallel <sup>12</sup>C-, <sup>13</sup>C-, <sup>12</sup>C<sup>14</sup>N- and <sup>12</sup>C<sup>15</sup>N- to measure the incorporation of <sup>15</sup>N (protein turnover). The results indicate that: 1. In the renal ischemia experiments replicated DNA can be imaged at high resolution directly from the <sup>81</sup>Br signal from incorporated BrdU. 2. Accumulation of <sup>15</sup>N from <sup>15</sup>N-leucine can be simultaneously measured and is both larger in the nuclei of cells that underwent DNA replication than in their cytoplasm and larger than in non-replicating cells (cytoplasm and nuclei). 3. Excellent histological details of the DNA were obtained from the mass <sup>81</sup>Br images. 4. One minute duration scans of 100x100 microns areas were enough to identify which nuclei were undergoing DNA replication. Thus, relatively large areas of sample may be observed rapidly to identify regions of interest for in-depth analysis.

## **References**

[1] J. Bonventre, Kidney ischemic preconditioning. *Curr. Opin. Nephrol. Hypertens.* 11(1) (2002), p.43-8.

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