Biomarkers of Ischemic Damage Associated with Liver Preservation Injury Prior to Transplant

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Introduction: Demand for liver transplant continues to increase while the donor organ pool remains limited. Post-transplant complications, such as early allograft dysfunction (EAD) can occur due to ischemic injury to the donor graft during liver preservation. There is a critical need to improve liver graft preservation methods to optimize post-transplant outcomes. Conventional static cold storage (SCS), or "on ice," preserves livers at 4^{o}C. Hypothermic Oxygenated Machine Perfusion (HMP-O_2) is a novel preservation technique, during which the liver is continuously preserved in a nutrient-rich oxygenated solution. Clinical data shows that HMP-O_2 improves post-transplant outcomes. The exact molecular pathways ameliorated by HMP-O_2 remain unknown. Herein we explore nuclear factor kappa B (NFκB), a transcription factor critical for the initiation of inflammatory responses, as a potential key player in preservation injury mitigated by HMP-O_2. **Methods:** Liver biopsies were obtained following the preservation period, during the transplant procedure (SCS N=6, HMP-O_2 N=6). Total and nuclear NFκB-p65 tissue expression was detected via immunohistochemistry, IHC (CellSignaling, 1:600). Staining density analysis was performed by two independent, blinded investigators, and results averaged between the two. Quantification of results was performed with Fiji ImageJ (NIH). Statistical analysis was performed via GraphPad Prism v9.4. A $p<0.05$ was considered significant. **Results:** There was decreased density of cytoplasmic staining of NFκB-p65 in livers preserved using HMP-O_2 compared to SCS ($p=0.04$). Similarly, nuclear translocation of NFκB was decreased in the HMP-O_2 cohort (9% vs 12%, p=ns). In subgroup analysis, patients with EAD post-HMP-O_2 showed increased parenchymal and nuclear NFκB staining compared to those without EAD ($p<0.0001$). Conversely, incidence of EAD did not affect tissue staining density of liver NFκB post-SCS. In this limited patient sample, there was no significant correlation between tissue parenchymal and nuclear staining ($r=0.3$, $p=0.4$). **Conclusions:** Livers preserved using HMP-O_2 showed decreased parenchymal and nuclear NFκB-p65 compared to SCS. Results did not show clear correlation between NFκB-p65 tissue density and incidence of EAD. Increased NFκB-p65 might represent an early preservation injury mediator. Early downregulation of NFκB might be a central pathway mitigated by HMP-O_2.

Keywords: liver preservation, static cold storage (SCS), hypothermic oxygenated machine perfusion (HMP-O_2), nuclear factor kappa B (NFκB), ischemic injury, early allograft dysfunction (EAD)