Analysis of Neutral Lipid Accumulation and Lipotoxicity in Non-hepatocyte Liver Cells Under Free Fatty-acid Treatment

Danielle Wilmes[1], Yujin Park[1], Kristine Farag[1], Ping Li[1], Wenjun Zhang[1*], Burcin Ekser[1*]

[1]Division of Transplant Surgery, Department of Surgery, Indiana University School of Medicine.
(*) contribute equally

BACKGROUND: Nonalcoholic fatty liver disease (NAFLD) and its advanced form, nonalcoholic steatohepatitis (NASH), are prevalent liver diseases with no effective treatment available. NAFLD is marked by increased lipid accumulation in the hepatocytes, lobular inflammation and fibrosis. However, the effects of lipid accumulation in other hepatic lineage cells, such as liver endothelial cells (LEC), intrahepatic cholangiocytes (IHCHOLs), and hepatic stellate cells (HSCs), on NAFLD/NASH progression are less understood.

METHODS: Human HSCs, IHCHOLs, and LECs were isolated from liver explants of healthy donors and further characterized with immunofluorescence staining. The cells were treated with different concentrations of free fatty acids (FFAs, oleic acid and palmitic acid 2:1) for 48 hours, and then examined for lipid accumulation with staining of BODIPY493/503. A cell viability assay and LDH cytotoxicity assay were performed. Real-time PCR was performed to monitor gene expression in LECs after treatment.

RESULTS: Lipid accumulation confirmed by BODIPY493/503 staining was observed following treatment, especially with higher FFA concentrations. No statistically significant difference in cell viability or cytotoxicity of the cells was observed under different treatment concentrations of FFA. However, a negative trend in cell viability was observed with an increment of FFA dosage. FFA treatment upregulated the expression of the genes in LEC that are related to angiogenesis (CDH5), epithelial-mesenchymal transformation (TGF-β2 and TGF-β3), cellular stress (P53), inflammation (IL-32) and immunoglobular cell adhesion molecules (ICAM-1 and VCAM-1) that are key to monocyte adhesion to endothelial cells in response to a local inflammatory signal.

CONCLUSION: Using an *in vitro* cellular model system, we examined lipid accumulation and cellular stress in different human liver cell types, especially LECs, under dose-dependent FFA treatment. This could be further developed to delineate adaptive responses within different hepatic lineage cells in the progression of NAFLD/NASH.

IMPACT: A better understanding of the involvement of hepatocytes and other hepatic lineages in the progression of NAFLD/NASH will augment the development of targeted and effective treatments.