Investigating the Effects of Tirzepatide on NAFLD/NASH Progression Using 3D Human Liver Organoids

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Background: Nonalcoholic fatty liver disease (NAFLD) is a disease characterized by the accumulation of lipids in the liver that ultimately progresses to nonalcoholic steatohepatitis (NASH) and cirrhosis. Currently, there is no known FDA-approved treatment for NASH. Tirzepatide (brand name Mounjaro), a glucagon-like peptide 1 (GLP-1) agonist, has been hypothesized to have potential effects in reversing NAFLD/NASH progression and restoring liver function. However, this hypothesis remains untested in current literature. The aim of this study is to use 3D human liver organoids (3D-HLOs) as an ex vivo model system to determine the potential effects of tirzepatide on the progression of NAFLD/NASH.

Methods: 3D-HLOs were constructed by incorporating 5 major human hepatic cell types isolated from NAFLD livers, including hepatocytes, hepatic stellate cells, liver endothelial cells, cholangiocytes, and Kupffer cells. NAFLD 3D-HLOs were maintained in the hepatocyte medium supplemented with 50mM free fatty acid (FFA) only (control group) or FFA+tirzepatide (100nM, 200nM, or 500 nM) for 14 days. By the end of the treatment, 3D-HLOs were subjected to BODIPY493/503 staining to determine lipid droplet accumulation. Immunofluorescence staining and confocal microscopy analysis were performed to confirm hepatocyte function (ALBUMIN) and fibrosis (COL1A1). qPCR was performed to determine the relative expression markers of hepatocyte function (ALBUMIN, HNF4A), angiogenesis (PECAM-1, VCAM-1, ICAM-1), and fibrosis (ACTA2, COL1A1).

Results: BODIPY 493/503 revealed no significant difference in lipid deposition between 3D-HLOs in all treatment groups. Mean immunofluorescence staining intensity for albumin in controls, 100nM, 200nM, and 500nM tirzepatide were 154.4, 181.4, 221.1, and 236.4, respectively ($p>0.05$). Dose-dependent quadratic regression revealed a strong correlation between dose and albumin fluorescence intensity ($R2 = 0.963$). qRT-PCR analysis revealed that tirzepatide treatment did not significantly alter transcriptional levels of ALB, HNFA4, ACTA2, PECAM-1, VCAM-1, and ICAM-1 compared to the control group.

Conclusion: Although there is no statistically significant difference in liver cell function markers at different tirzepatide dosages in the setting of NAFLD/NASH, the highest dose of tirzepatide improved ALB expression despite ongoing FFA exposure, indicating a potential hyperbolic relationship between tirzepatide concentration and albumin production. Future investigation with altered treatment duration and dosage will elucidate whether there are any direct modulating effects of tirzepatide on NAFLD/NASH progression.

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