Utilization of exVive3D™ Human Liver Tissues for the Evaluation of Valproic Acid-Induced Liver Injury

Candace Grundy, Ryan Smith, Jeff Nickel, Rhiannon N. Hardwick, and Deborah G. Nguyen | Organovo, 6275 Nancy Ridge Drive Suite 110, San Diego, CA 92121

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Abstract

Conventional (2D) cell culture models do not accurately reflect the complex microenvironment of liver tissue, and pre-clinical animal trials are often inadequate due to species-specific variation in hepatocellular functions. 3D bioprinted human liver tissues better approximate human tissue composition and physiology, and therefore enable the assessment of drug-induced liver injury (DILI) and related mechanisms at the tissue level, including biochemical and histologic outcomes. In this study, we assessed the DILI response to Valproic Acid (VPA), a compound known to induce steatosis in humans, in 3D-bioprinted human liver tissue mimetics comprised of primary hepatocytes, hepatic stellate cells, and endothelial cells. 3D-bioprinted human liver tissues (exVive3D, Organovo, San Diego CA), were treated daily for 14 days with VPA, which resulted in dose-dependent decreases in tissue health as assessed by ATP, GSH, and histology. Tissue ATP levels were decreased 70% and 45% relative to vehicle following 14 day treatment with 1mM and 5mM VPA, respectively. Following the observations seen with ATP levels, the higher doses of 200µm, 1mM, and 5mM were selected to further evaluate the mechanism of observed tissue damage. GSH levels were measured at 24hr and 72hr to determine the acute oxidative stress response. A significant decrease in the ratio of reduced to oxidized GSH was observed at 24hr treatment, indicating increased oxidative stress. Recovery of GSH ratios was noted with 200µm and 1mM treatment groups at 72hr, while the 5mM treatment group exhibited prolonged oxidative stress. Histological evaluation of tissues revealed dose-dependent damage with abundant vacuolization in 5mM VPA treated tissues, consistent with a steatotic phenotype. Collectively, these data suggest exVive3D human liver tissues can be used to investigate the mechanisms of DILI *in vitro*, as evidenced by the expected results seen in both biochemical and histologic endpoints following treatment with VPA.

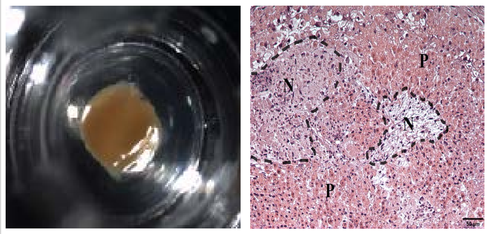


Figure 1: (Left) Representative image of single exVive3D human liver tissue, measuring 2.5 x 2.5mm, with a 0.5mm thickness. Representative images of H&E section (Right) showing distinct zones of non-parenchymal (N) and parenchymal (P) cells.

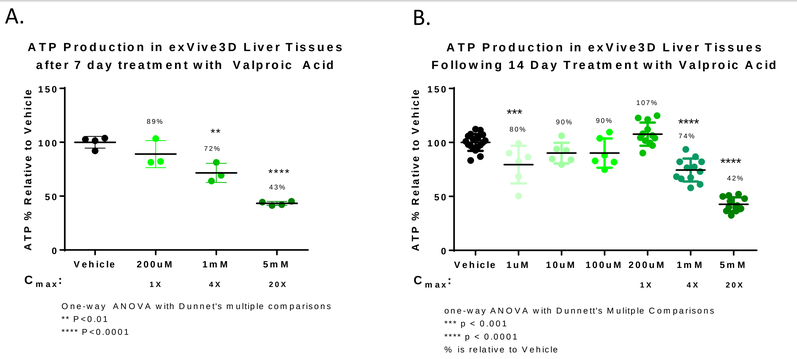


Figure 2: exVive3D Human Liver tissues effectively model dose-dependent VPA induced liver injury. exVive3D human liver tissues were treated with vehicle or VPA daily for up to 14 days. Viability was assessed by ATP measured in tissue homogenates (Promega, Cell Titer Glo™) following 7 (A) and 14 days (B) of treatment. Decreased tissue viability was observed after 7 days of treatment with the higher doses of VPA and persisted in tissues treated for 14 days. Hepatocyte health was assessed by measuring albumin (C) in supernatants (Bethyl, Inc.) following 7 or 14 days of treatment. Albumin production decreased dose and time dependently with significant decreases in tissues treated with sub C_{max} doses for 14 days. Data shown is the mean +/- standard deviation for at least 14 independent tissue replicates.

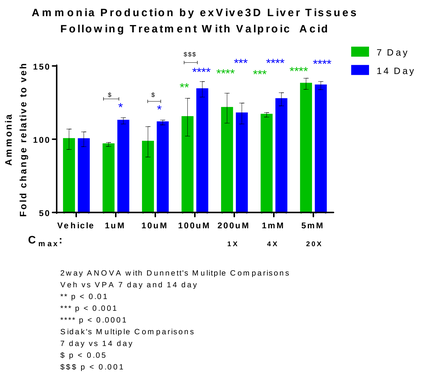


Figure 3: exVive3D Human Liver tissues effectively demonstrate VPA stimulated ammonia production. Increased ammonia production was consistent with hyperammonemia which is associated with clinical exposure to VPA. Dose and time dependent increases in ammonia production were observed. Data shown is the mean +/- standard deviation for at least 4 independent tissue replicates.

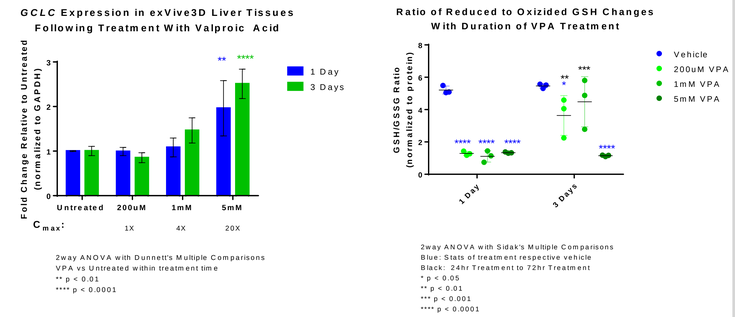


Figure 4: exVive3D Human Liver tissues model oxidative stress leading to tissue damage. A) exVive3D Human Liver tissues treated with VPA have increased *GCLC* (glutamate-cysteine ligase, catalytic subunit) expression, the enzyme responsible for glutathione synthesis, as early as 24hrs after 5mM treatment. B) GSH levels measured in tissue homogenate (GSH/GSSG-glo™) also indicate tissues are under oxidative stress following 24hr treatment. Oxidative stress persists following 3 days of treatment in tissues treated with 5mM VPA, with partial recovery at lower doses. Data shown is the mean +/- standard deviation for 3 independent tissue replicates.

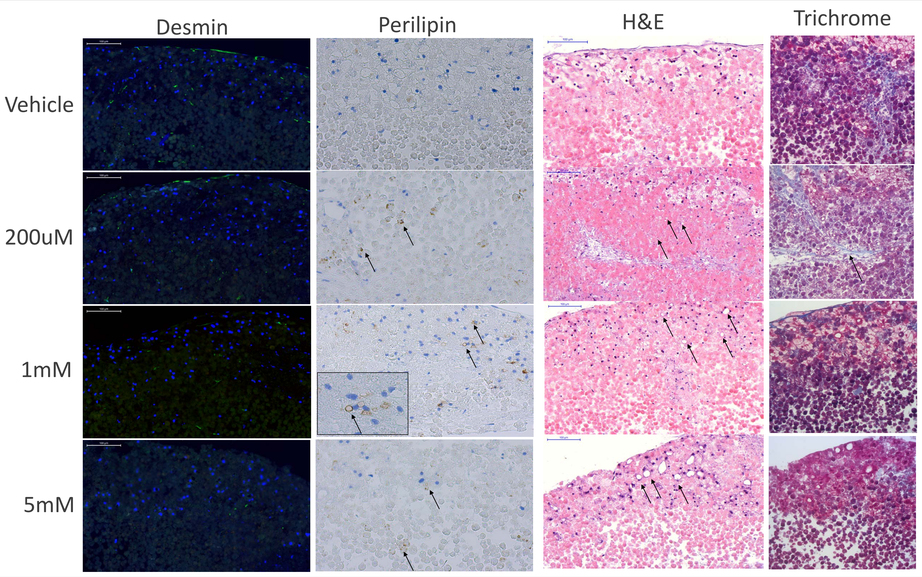
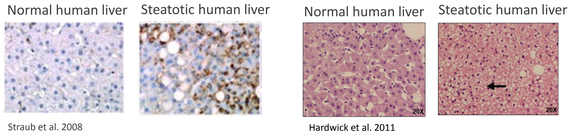


Figure 5: exVive3D Human Liver tissues model the histopathologic damage associated with chronic VPA treatment. The presence of desmin+ stellate cells (green) decreased with increasing concentrations of VPA, suggesting loss and/or activation of stellate cells. Perilipin staining of lipid (brown indicated by arrows) within the tissues increased with increasing VPA concentration consistent with a steatotic phenotype. Immunodetection of perilipin+ cells was most notable in 1mM (4x C_{max}) VPA treated tissues, localized to the margins of large vacuoles. This steatotic phenotype (indicated by arrows) is further evidenced in the H&E images which show dose-dependent damage with abundant vacuolization in 5mM VPA treated tissues. In parallel with the observed decrease in desmin+ stellate cells, increased collagen deposition was evident with Gomori's Trichrome, in the 200uM treated tissues, consistent with the development of fibrosis. Higher doses of VPA resulted in dissociation of cells, apparent as individualized cells with little to no intercellular junctions, and cell death.



Straub et al. 2008

Hardwick et al. 2011

Summary

exVive3D Liver tissues effectively model multiple modes of VPA-induced liver injury:

Viability and Hepatocyte Health

- exVive3D Liver tissue viability decreased dose-dependently with sub C_{max} doses having no effect, while 4 and 20x C_{max} significantly decreased tissue viability.
- Albumin production also diminished dose dependently. 14 day treatment resulted in significant decreases in concentrations well below C_{max}.
- These data suggest loss of hepatocyte function (albumin production) precedes significant loss of tissue viability.

Ammonia Production

- exVive3D Liver tissues demonstrated dose dependent increases in ammonia production.
- 14 day treatment resulted in significantly increased production compared to 7 day.
- These data are consistent with increased ammonia production associated with clinical exposure to VPA.

Oxidative Stress

- exVive3D Liver tissues had increased expression of GSH related *GCLC*.
- Ratios of GSH and GSSG production decreased following VPA treatment.
- Taken together these data suggest that the tissues are in a state of oxidative stress, a known mechanism of liver injury following VPA treatment.

Histopathologic Damage Associated with Chronic Valproic Acid Treatment

- exVive3D Liver tissues treated with 200uM VPA have decreased desmin+ cells with increased trichrome staining, consistent with a fibrotic phenotype.
- Tissues treated with higher doses of VPA (1mM and 5mM) have apparent dissociation of tissue mass via loss of cell-cell junctions.
- H&E and perilipin staining of exVive3D Liver tissues following 14 day treatment reveal histology consistent with microvesicular (lower doses) and macrovesicular (higher doses) steatosis.