Utilization of the ExVive Human Liver Tissue Model to Assess Drug-Induced Liver Injury across a Diverse Set of Chemical Classes

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Abstract

One of the key challenges in the drug development process continues to be the early identification of compounds with adverse and potentially dose-limiting liver toxicity. Traditionally hepatotoxicity prediction has relied on two-dimensional hepatocyte monolayers, sandwich culture assays and non-human animal models. These systems are limited in their ability to accurately reflect in vivo human biology and lack of the cellular complexity required to model tissue-level outcomes of toxicity. In this study, drug-induced liver injury (DILI) was assessed in 3D-bioprinted human liver tissues comprised of primary hepatocytes, hepatic stellate cells, and endothelial cells (ExVive™ Human Liver Tissues) treated with known high and low DILI risk compounds. Tissue response to compounds was evaluated using a range of biochemical, cytokine secretion, gene expression and histologic analyses. The high DILI risk compounds tolcapone, benzbromarone, danazol and tamoxifen were evaluated using a 28 day dosing regimen and compared to safer compounds entacapone, phentolamine, betahistine, nifedipine and chloramphenicol. Tissues treated with the known toxicants exhibited evidence of toxicity in at least two assays. A comparison of the clinically related compounds tolcapone and entacapone at concentrations of 1x, 3x and 10x C_{max} revealed clear differences in their impact on the bioprinted tissues; tolcapone resulted in a dose dependent decrease in tissue viability at 10x C_{max} while entacapone resulted in no significant changes in viability. Significant reductions in albumin secretion were seen at 3x C_{max} with tolcapone treatment, vs. 10x C_{max} with entacapone. Treatment of the 3D liver tissues with 5x and 20x C_{max} concentrations of benzbromarone for 28 days resulted in decreased tissue viability with the highest concentration along with time and dose dependent decreases in albumin production beginning at treatment day 7 with the 5x and 20x C_{max} concentrations. Histologic assessment of these tissues revealed significant loss of tissue and disruption of cellular cohesion at the 20x C_{max} dose. These results suggest 3D bioprinted liver tissues are well suited to differentiate high risk from low risk DILI compounds and utilize both biochemical and histologic endpoints to assess multiple mechanisms of DILI in vitro, providing a comprehensive means of examining tissue injury.

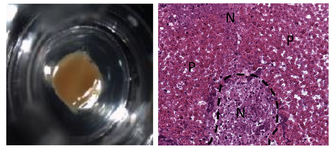

Figure 1: (Left) Representative image of single ExVive Human Liver Tissue, measuring 2.5 x 2.5mm, with a 0.5mm thickness. Representative image of H&E section (Right) showing distinct zones of non-parenchymal (N) and parenchymal (P) cells.

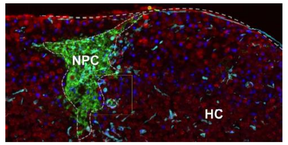

Figure 2: Representative immunofluorescence image of section showing distinct zones of non-parenchymal (NPC) in green and parenchymal (HC) cells in red.
Norona, et al. (2016) Tox Sci. Dec;154(2):354-367.

Tolcapone and Entacapone

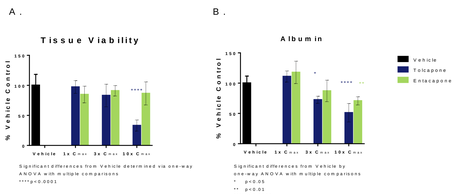

Figure 3: ExVive Human Liver Tissue is able to show differential in response to treatment with Tolcapone, which was withdrawn due to liver tox, and the safer alternative Entacapone. ExVive Human Liver Tissues were treated with vehicle, Tolcapone or Entacapone daily for up to 28 days. Viability was assessed by ATP measured in tissue homogenates (Promega, Cell Titer GloTM) following 28 days (A) of treatment. Decreased tissue viability was observed in tissues treated with Tolcapone but not with Entacapone treatment, indicating Tolcapone treatment resulted in damage to the tissues while Entacapone did not impact over all tissue viability. Hepatocyte health was assessed by measuring albumin (B; Bethyl) in supernatants following 28 days of treatment. Dose dependent decreases in Albumin were observed with both Tolcapone and Entacapone treatment indicating impacts to hepatocyte health, but to a lesser degree with Entacapone. Data shown is the mean +/- standard deviation for at least 5 independent tissue replicates.

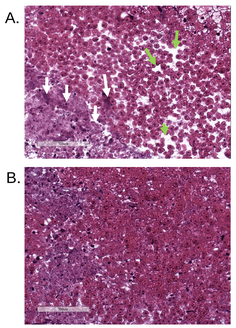

Figure 4: ExVive Human Liver Tissue is able to show differential histological phenotypes in response to treatment with Tolcapone. Histologic observations indicate areas of cell death (white arrows) with tissue dissociation and loss of cell-cell junctions (green arrows) following 14 days of treatment with high dose Tolcapone (A), while tissues treated with Entacapone show no damage (B).

Perhexiline

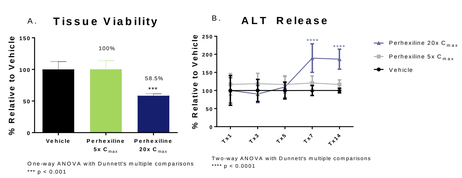

Figure 5: ExVive Human Liver Tissue is able to model Perhexiline induced toxicity. ExVive Human Liver Tissues were treated with vehicle, or Perhexiline daily for up to 14 days. Viability was assessed by ATP measured in tissue homogenates (Promega, Cell Titer Glo™) following 14 days (A) of treatment. Decreased tissue viability was observed in tissues treated with 20x C_{max} Perhexiline, indicating decreased tissue viability. Hepatocyte injury marker, ALT was assessed (B) in supernatants (Biotang) with hepatocellular damage indicated by increased ALT release observed at both Tx7 and Tx14. Data shown is the mean +/- standard deviation for at least 4 independent tissue replicates.

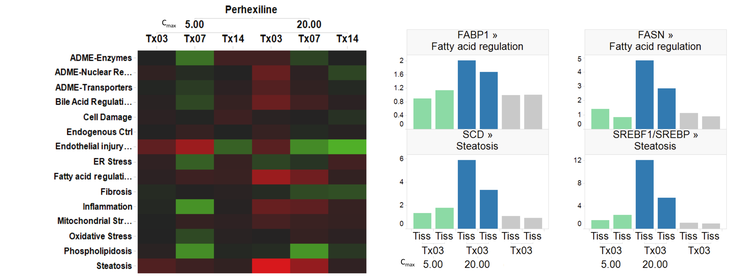

Figure 6: ExVive Human Liver Tissue is able to model Perhexiline induced toxicity with indications to mechanism of injury. ExVive Human Liver Tissues were treated with vehicle, or Perhexiline daily for 3 days. Using a panel of liver injury related gene targets, changes in expression levels were evaluated following treatment to elucidate potential mechanisms of action. Genes related to fatty acid and steatosis pathways, were found to be upregulated, consistent with the known mechanism affecting fatty acid metabolism. [Jaeschke et al, Toxicol Sci (2002)]

Benzbromarone

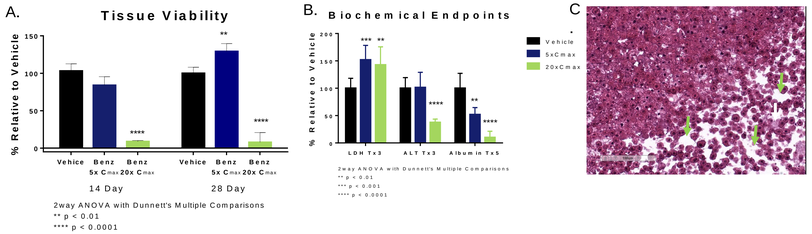

Figure 7: ExVive Human Liver Tissue is able to model Benzbromarone induced toxicity. ExVive Human Liver Tissues were treated with vehicle, or Benzbromarone daily for up to 28 days. Viability was assessed by ATP measured in tissue homogenates (Promega, Cell Titer Glo™) following both 14 and 28 days (A) of treatment. Decreased tissue viability was observed in tissues treated with 20x C_{max} Benzbromarone, indicating decreased tissue viability. Biochemical injury markers, LDH, ALT and albumin were assessed (B) in supernatants (Abcam, Biotang, and Bethyl, Inc.) with cell damage indicated by increased LDH release and decreased ALT observed early at Tx3, and a dose dependent reduction in hepatocyte health observed in albumin production by Tx5. Data shown is the mean +/- standard deviation for at least 5 independent tissue replicates. Histologic observations (C) indicate areas of cell death (white arrows) with tissue dissociation (green arrows) following just 14 days of treatment.

Danazol and Tamoxifen

Figure 8: ExVive Human Liver Tissue is able to model toxicity associated with Danazol and Tamoxifen, which are known to cause liver injury. ExVive Human Liver Tissues were treated with vehicle, Danazol, or Tamoxifen daily for up to 28 days. Hepatocyte health was assessed by measuring ALT (A) in supernatants (Biotang) over 7 days of treatment. Significantly increased ALT release was observed following just 3 days of treatment with 1x and 20x C_{max}, with Danazol, and following Tx7 with 5x and 20x C_{max} of Tamoxifen, indicating impacts to hepatocyte health. Data shown is the mean +/- standard deviation for at least 5 independent tissue replicates. Cytokine production (MesoScale) was assessed following 3 days of treatment (B). Production of IL-1β was significantly induced following treatment with Danazol, likely from the NPCs with 20x Cmax treatment, no changes to cytokines were observed with Tamoxifen.

Negative Controls: Phentolamine, Betahistine, Nifedapine and Chloramphenicol

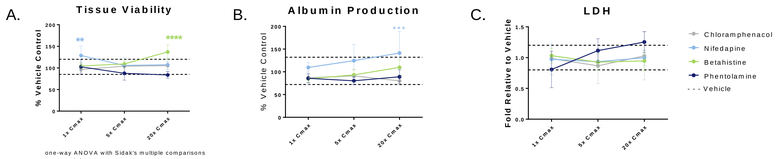

Figure 9: ExVive Human Liver Tissue is able to show no associated toxicity following treatment with compounds not associated with liver toxicity. ExVive Human Liver Tissues were treated with vehicle, Phentolamine, Betahistine, Nifedapine, or Chloramphenicol daily for up to 28 days. Viability was assessed by ATP measured in tissue homogenates (Promega, Cell Titer Glo™) following 28 days (A) of treatment. Treatment did not adversely impact overall tissue viability. Hepatocyte health was assessed by measuring albumin (B) in supernatants (Bethyl, Inc.) following 28 days of treatment. Treatment did not result in any detrimental impacts to hepatocyte health. LDH Release (C) was also measured following 7-days of treatment to assess if any cell damage has occurred. There were no significant increases in LDH release indicating no cell damage has occurred with treatment. Data shown is the mean +/- standard deviation for at least 5 independent tissue replicates, dashed lines indicated vehicle standard deviation.

Table 1: ExVive Human Liver DILI Summary of Results

Compound	DILI Rank[5]	Injury	C_{max}	ExVive DILI Result
Tolcapone	Severe	Metabolic / Mitochondrial	11μM[4]	Positive
Entacapone	Low	Metabolic/Paired Control	4μM[2]	Positive
Benzbromarone	Severe	Metabolic / Mitochondrial	4.4μM[1]	Positive
Danazol	Severe	Cholestatic/Hepatocellular Carcinoma/ Hepatic Adenoma	74nM[1]	Positive
Tamoxifen	Severe	Fatty liver, Steatohepatitis	162nM[1]	Positive
Perhexaline	Severe	Mitochondrial/ Steatosis	280nM[3]	Positive
Phentolamine	None		86nM[1]	Negative
Betahistine	None		4nM[1]	Negative
Nifedipine	Low	Toxic Intermediate	271nM[1]	Negative
Chloramphenicol	None		20μM[1]	Negative

C_{max} values obtained from [1]Khetani et al. 2013; [2]Oorts et al, 2016; [3] Porceddu et al. 2012; [4]http://www.accessdata.fda.gov/drugsatfda_docs/nda/98/20697_Tasmar_pharmr_P1.pdf
[5] DILI rank compiled from multiple sources: livertox.nih.gov/; Chen et al, 2016; Khetani et al. 2013; Gustafsson et al. 2014; Schadt et al. 2016

Summary

ExVive Human Liver Tissue is able to detect DILI of known toxicants using measures of viability, and biochemical markers such as LDH, albumin, and ALT. In addition, these tissues can be used to evaluate potential mechanisms of injury using cytokine release and gene expression analysis with a panel of liver injury targets. Histologic assessment following treatment also provides the ability to look at morphological changes in the tissues to aid in elucidating mechanisms of action.

These results demonstrate the ability of ExVive Human Liver Tissue to:

- **Differentiate between toxic and non-toxic compounds.**
- **Assess toxicity using both standard biochemical markers and additional mechanistic readouts (cytokines, gene expression) to assess potential mechanism of action**
- **Model cell type-specific damage (ex: tamoxifen effects on albumin and ALT without impacting global viability)**
- **Differentiate between similar compounds with different degrees of toxicity (ex: Tolcapone vs Entacapone)**

Forward-Looking Statements
This presentation contains statements about future events and expectations known as "forward-looking statements" within the meaning of Section 27A of the Securities Act of 1933, as amended (the "Securities Act"), and Section 21E of the Securities Exchange Act of 1934, as amended (the "Exchange Act"). The Company has based these forward-looking statements on its current expectations and the information currently available to it, but any forward-looking statements are subject to a number of risks and uncertainties. The factors that could cause the Company's actual future results to differ materially from its current expectations, or from the results implied by any forward-looking statements, include, but are not limited to, risks and uncertainties relating to the Company's ability to develop, market and sell products and services based on its technology; the expected benefits and efficacy of the Company's products, services and technology; the Company's ability to successfully complete studies and provide the technical information required to support market acceptance of its products, services and technology, on a timely basis or at all; the Company's ability to generate revenue and control its operating losses; the validity of the Company's intellectual property rights and the ability to protect those rights; the Company's ability to implement and achieve its business, research, product development, regulatory approval, marketing and distribution plans and strategies; the Company's ability to secure additional contracted collaborative relationships; and the Company's ability to meet its fiscal-year 2017 outlook and/or its long-range outlook. These and other factors are identified and described in more detail in the Company's filings with the Securities and Exchange Commission ("the SEC"), including those factors listed under the caption "Risk Factors" in the Company's Form 10-K for the year ended March 31, 2016, filed with the SEC on June 9, 2016, as well as other filings Organovo makes with the SEC from time to time.

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