A human in vitro three-dimensional bioprinted liver tissue system can be used to model nutritional damage and protective effects of MSDC-0602K, a novel modulator of the mitochondrial pyruvate carrier (MPC).

Jerry R. Colca[1], Dwayne E. Carter[2], Andrea S. Rothmeier[2], Thomas J. Murphy[2] and Alice E. Chen[2]

[1]Cirius Therapeutics, Kalamazoo, MI and San Diego, CA

[2]Organovo Inc, San Diego, CA

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INTRODUCTION

The growing global incidence of NASH mirrors the availability of nutrients. Over-nutrition also results in insulin resistance and type-2 diabetes, which are often co-morbidities associated with NASH and are known to drive more adverse outcomes.

MSDC-0602K, a modulator of the mitochondrial pyruvate carrier (MPC), is in clinical trials as a potential treatment for NASH. Preclinical studies have shown that the mitochondrial pyruvate carrier is increased in expression in animals fed a high fat diet. Moreover, selective knockouts of each of the mitochondrial proteins that make up the carrier have shown that the MPC is a key driver of both NASH pathology and pharmacology of MSDC-0602K.

Diet-induced disease using a three-dimensional multicellular human tissue model provides the potential to reconstruct the effects of overnutrition *in vitro* and to potentially model the actions of an agent like MSDC-0602K.

AIM

To determine whether bioprinted human liver tissue can be used to model the pathology produced by over nutrition.

To characterize the ability of MSDC-0602K to modulate disease phenotype in a human relevant model of NASH.

To further the understanding of the pharmacology of MSDC-0602K and provide evidence for non-invasive biomarkers that might predict clinical response.

METHODS

ExVive™ Human Liver Tissue (Organovo, San Diego) was fabricated by bioprinting primary human hepatocytes, hepatic stellate cells, Kupffer cells, and endothelial cells into a 3D tissue architecture. Tissues matured *in vitro* for three days and then were challenged with various concentrations of simple sugars and fatty acids. MSDC-0602 (Cmax clinical levels) administered either in tandem with nutrient overload (Time = 0hrs) or one week following initiation of nutrient overload (Time = Week 1). Tissues were stained for α-smooth muscle actin for stellate cell activation and Masson's Trichrome for content of collagen. Medium was collected for assessment of hepatocyte function (Albumin), and potential markers of tissue (Alanine Transferase, ALT) and mitochondrial (mitochondrial DNA, MT-ATP6) damage.

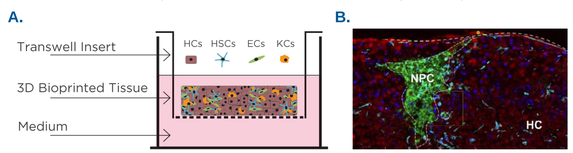
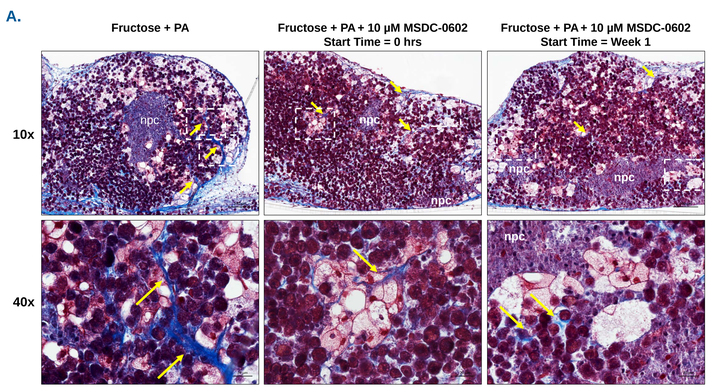


Figure 1. **[A]** Schematic of ExVive™ Human Liver Tissue, comprised of primary human hepatocytes (HCs), hepatic stellate cells (HSCs), and endothelial cells (ECs) bioprinted in a compartmentalized geometry onto the membranes of 24-well transwell culture inserts. **[B]** Representative immunofluorescence image of 3D human liver tissue showing distinct zones of non-parenchymal cells (NPC) in green and parenchymal (HC) cells in red. (*Norona, et al. 2016*)

RESULTS



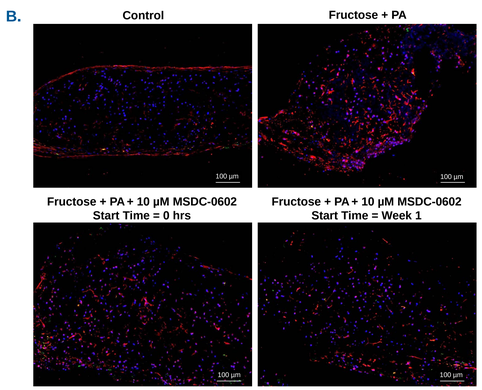
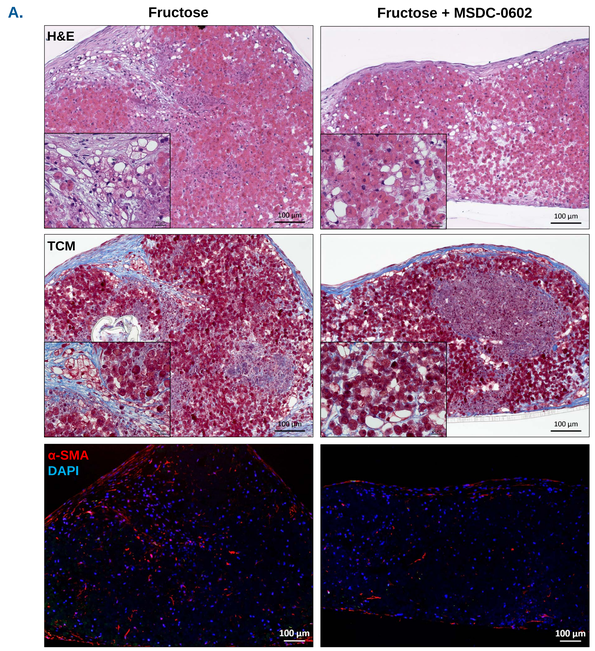
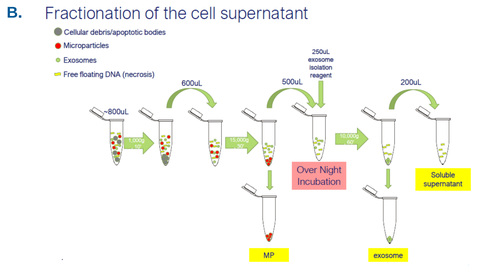


Figure 2. **[A]** The combination of fructose and fatty acid stimuli palmitate (PA) produced NASH-type liver pathology including steatosis, inflammation, ballooning, and fibrosis. Collagen deposition and fibrosis (yellow arrows) were reduced by adding MSDC-0602 either in parallel with or up to one week after the challenge. **[B]** Addition of MSDC-0602 was also associated with a reduction in stellate cell activation following disease induction (smooth muscle actin – red).





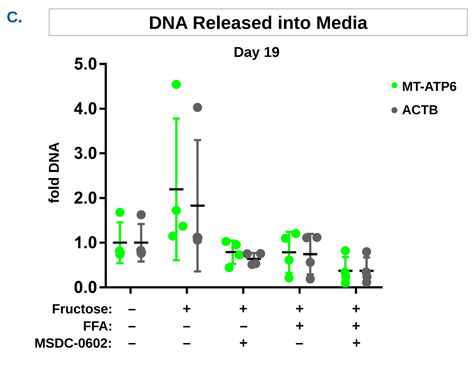


Figure 3. In a repeat study, fructose was either added alone or with combination of fatty acids (FFA). The addition of 10 µM MSDC-0602 was made three days after the nutrient stimulus. At the end of the experiment, medium was analyzed for nuclear and mitochondrial DNA. **[A]** Tissues treated with fructose + MSDC-0602 exhibited reduced collagen deposition and fibrosis near areas of hepatocyte injury as assessed by H+E and trichrome stains as well as reduced α-SMA expression. **[B]** Circulating hepatocyte-derived microparticles (MP) that contain high amounts of mitochondrial DNA (mtDNA) have emerged as a potential biomarker for the metabolic insult in NASH patients (*Garcia-Martinez, et al. 2016*). Extracellular vesicles were collected from the tissue supernatants and **[C]** analyzed for mtDNA and nuclear DNA (nDNA), by qPCR of the mitochondrial MT-ATP6 gene and nuclear ACTB gene.

CONCLUSIONS

These data show that human 3D bioprinted liver tissue can be adapted for demonstrating NASH-type liver pathology and the pharmacology of a novel MPC modulator can be modeled in this system.

- Addition of sugars and fatty acids are sufficient to induce steatosis and tissue damage including putative hepatocyte ballooning, stellate cell activation, and fibrosis.
- The addition of 10 µM MSDC-0602 either before or after initiation of the nutrient-stimulated damage showed protective effects against disease progression.
- Stimulating the bioprinted liver tissue with fructose alone was also associated with the induction of NASH-like pathology. Treatment with MSDC-0602 + Fructose reduced the prevalence of stellate cell activation and subsequent tissue fibrosis.
- Both mitochondrial DNA and nuclear DNA are released to the medium. Conditions are being further optimized to determine if there are specific release of markers indicative of nutrient induced damage.

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PHASE 2B CLINICAL TRIAL: EMMINENCE™ (ID: NCT02784444)

- 52-week evaluation of 3 exposures of MSDC-0602K versus blinded placebo in subjects with biopsy confirmed NASH
- Once-daily oral dosing
- 45 US sites
- At least 380 subjects to be enrolled