Monocrotaline Toxicity in 3D-Bioprinted Human Liver Tissue

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ABSTRACT: Monocrotaline (MCT), a pyrrolizidine alkaloid causes liver injury in animals similar to that of hepatic venoocclusive disorder in humans. MCT induced liver injury occurs through a complex set of cellular insults involving multiple cell types which can ultimately lead to fibrotic changes. In this study, we evaluated the effects of MCT in 3D-bioprinted human liver tissue comprising of primary hepatocytes, hepatic stellate cells, and endothelial cells (exVive3D™; Organovo, San Diego CA). The bioprinted tissues were treated with MCT at concentrations of 0.02, 0.2 or 2 mM for seven days. MCT treatment led to time- and dose-dependent decreases in tissue health as measured by LDH leakage and albumin synthesis and by histopathologic changes in the tissues. A dose-dependent increase in soluble LDH and a decrease in albumin production was observed as early as 3 days of treatment with MCT. Additionally, on treatment day 3, increases in the production of the pro-inflammatory cytokines IL-1b, IL-4, IL-8 and IL-10 were observed. Histologic assessment of formalin-fixed, paraffin-embedded tissue sections on treatment day 7 revealed early signs of tissue damage, including dissociation of the network of hepatocytes and reduced cellularity within the tissues. Immunohistochemical analyses revealed a dose-dependent increase in CD31⁺ cells and a marked increase in the appearance of large, CD31+ bright cells, often forming clusters or complex multi-cellular structures and cells co-expressing desmin and alpha smooth muscle actin (SMA). Changes in organization of CD31 expressing endothelial cells and appearance of desmin and SMA coexpressing cells are indicative of remodeling and initiation of fibrotic events. These observations capture the spectrum of changes induced by MCT ranging from reduced hepatocellular function and vascular remodeling, which may involve endothelial cell migration, organization, proliferation, stellate cell activation and initiation of early fibrotic events.

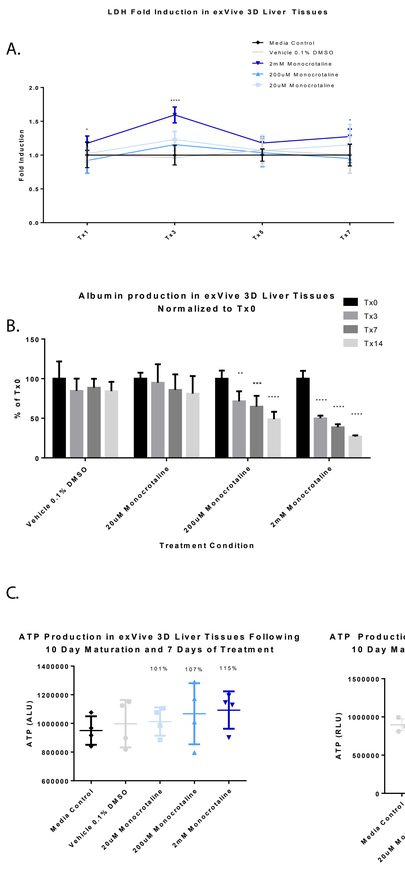

FIGURE 1. Biochemical analysis of media and 3D bioprinted human liver tissue exposed to monocrotaline for either 7 or 14 days. (A) LDH release (B) Albumin production (C) Whole tissue ATP levels. Monocrotaline treatment did not significantly affect viability of liver tissue as measured ATP levels, however, a transient increase in LDH was observed on treatment day 3. Monocrotaline treatment significantly affected the function of liver tissue as noted by decreases in albumin synthesis.

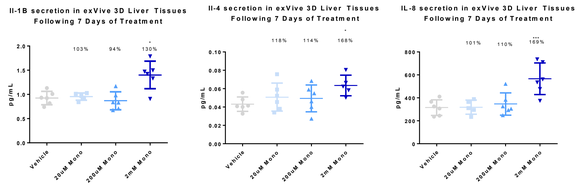

FIGURE 2. Dose dependent increases in cytokines observed following monocrotaline treatment. Cytokines were analyzed following treatment day 7 using an MSD pro-inflammatory 10-spot assay

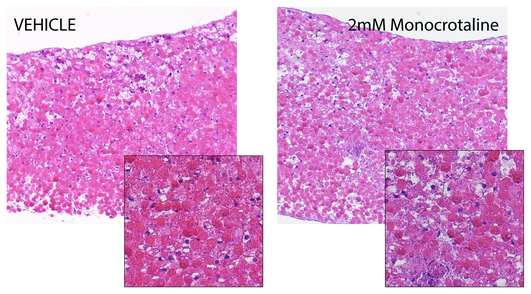

FIGURE 3. H&E stained tissues exposed to vehicle or 2mM monocrotaline. Monocrotaline treatment was associated with dissociation of cellular network and what appears to be a mild reduction in cellularity overall. Top row 100x, bottom row 200x magnification.

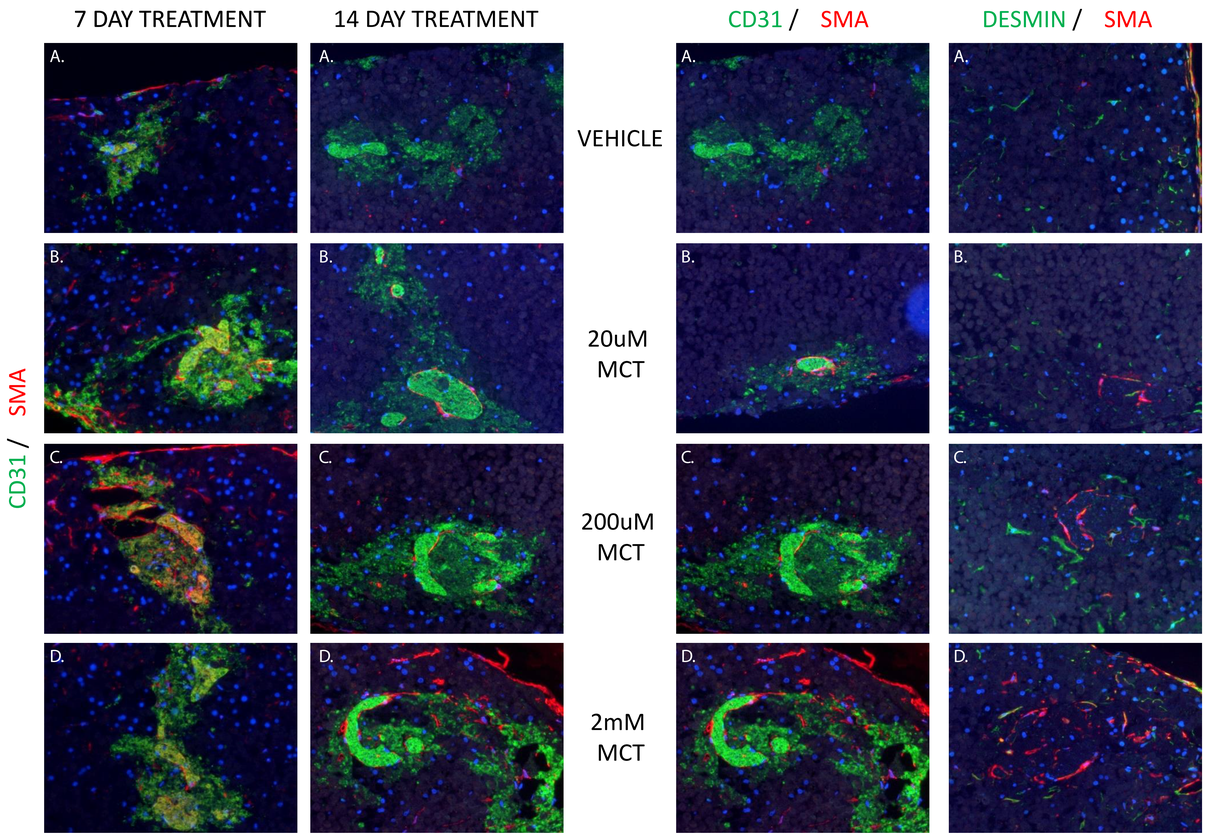

FIGURE 4. Alpha smooth muscle actin (SMA, marker of fibrosis); red) co-staining with CD31 (marker of endothelial cells; green) following seven or 14 days of treatment. (A) Vehicle treatment (B) 20uM MCT (C) 200uM MCT (D) 2mM MCT. Monocrotaline treatment resulted in an overall increase in CD31+ staining. In addition, at higher concentrations, increases in larger and brighter CD31+ structures were observed following 7 day treatment that co-stained for SMA. By 14 days we observed distinct staining of the CD31 and SMA markers. These observations are indicative of endothelial remodeling.

FIGURE 5. Alpha smooth muscle actin (SMA, marker of fibrosis); red) co-staining with either CD31 (marker of endothelial cells; green) or Desmin (marker of stellate cells; green) following 14 days of treatment. (A) Vehicle treatment (B) 20uM MCT (C) 200uM MCT (D) 2mM MCT. Monocrotaline treatment resulted in an increase in SMA staining in the tissue, with SMA staining adjacent to the large CD31+ structures and overlapped with the Desmin+ cells. These observations are suggestive of onset of tissue remodeling involving both non-parenchymal cells types in the tissue and initiation of stellate mediated pro-fibrotic events.

SUMMARY: The mechanism of monocrotaline induced liver toxicity is complex and involves multiple pathways and cell types. In this study we used a 3D-bioprinted human liver tissue model (comprising of primary hepatocytes, hepatic stellate cells, and endothelial cells) to demonstrate the spectrum of changes induced by monocrotaline. Monocrotaline treatment led to:

- Time- and dose-dependent decreases in tissue health as measured by albumin production
- Increases in production of pro-inflammatory cytokines IL-1b, IL-4, IL-8 and IL-10
- Histologically, signs of tissue damage, including dissociation of the network of hepatocytes and reduced cellularity
- Dose-dependent increase in CD31+ cells and a marked increase in the appearance of large and bright CD31+ staining cells, often forming clusters or complex multi-cellular structures
- Dose-dependent changes in organization of CD31 expressing endothelial cells and appearance of desmin and SMA co-expressing stellate cells indicative of tissue remodeling and initiation of early fibrotic events

These observations indicate the utility of the 3D tissue model to capture complex multi-cellular events that are not typically captured by traditional 2D in vitro systems.