Investigating Small Molecule Liver Toxicity by Matrix-Assisted Laser Desorption Ionization-Image Mass Spectrometry (MALDI-IMS)

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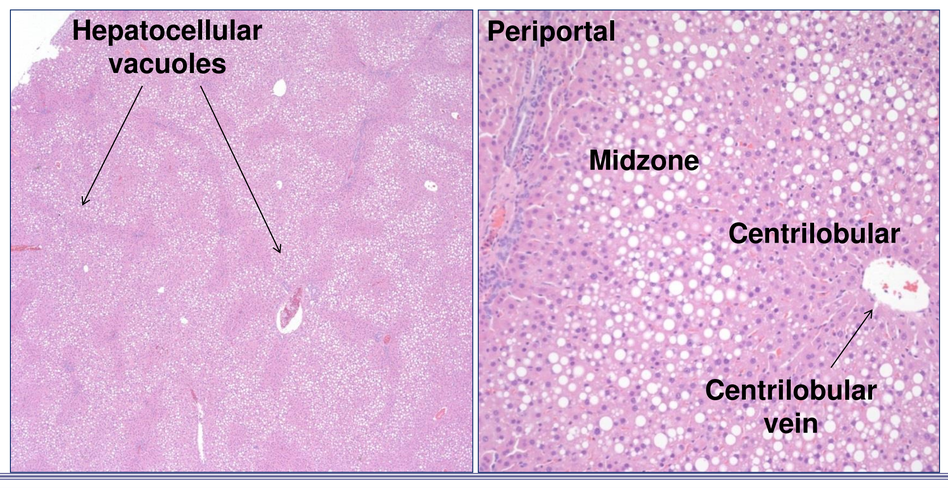
Background

Drug biodistribution in preclinical safety studies is routinely evaluated by using whole body autoradiography (WBA) or positron emission tomography (PET). However, these methods require radiolabeling and do not provide information on the drug metabolites. Other methods like Liquid Chromatography Mass Spectrometry (LC-MS) can identify compounds from tissue but require homogenization and, thereby, do not preserve tissue morphology. MALDI-IMS is an emerging label-free approach that enables *in situ* detection of drugs and their metabolites as well as lipids, proteins and peptides by direct analysis of fresh frozen tissue sections. Herein, we describe the use of MALDI-IMS to investigate small molecule drug localization in the context of liver histomorphological changes.

Small molecule-induced midzonal to centrilobular *hepatocellular vacuolation* in a 14 day rat study:



Objectives

- To investigate the potential co-localization of small molecule parent compound and/or its metabolites within hepatic vacuoles (morphologically consistent with lipid) in the frozen liver samples
- To explore any differential hepatic zonal distribution and/or composition of lipids associated with the vacuolation pattern

MALDI-IMS Workflow and Study Design

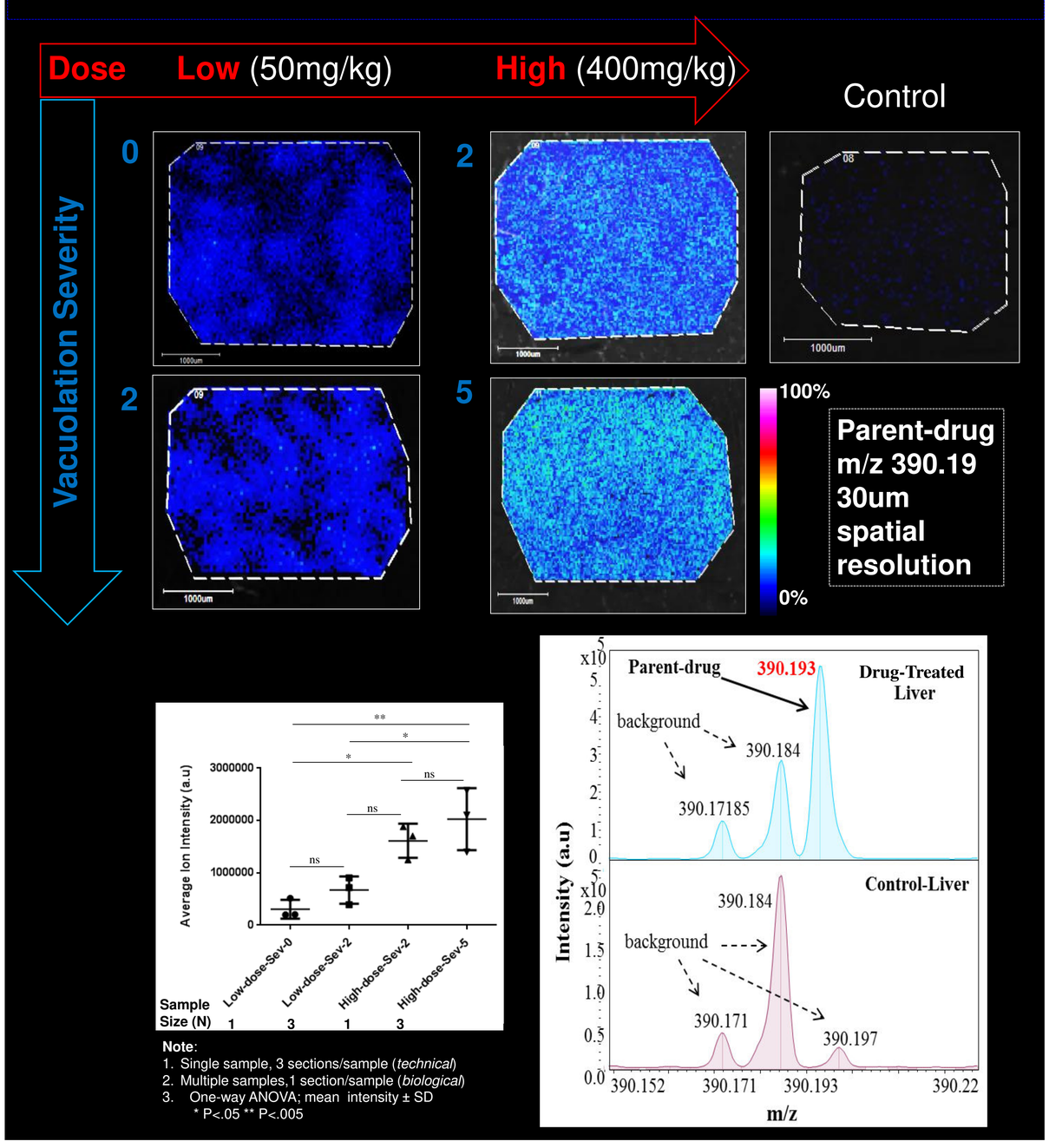
Frozen Section
⬇
Aqueous Buffer Wash
⬇
Matrix Deposition (1,5 diaminonapthalene, DAN) (*Sublimation*)
⬇
Laser Desorption/ Ionization/Detection
⬇
Data Acquisition, Processing and Analysis
⬇
Histological correlation

Dose Group 5 animals/ group	Dose Level	Vacuolation Severity Score Range*
Control	0 mg/kg	None
Low	50 mg/kg	0 to 5
Mid	150 mg/kg	3 to 4
High	400 mg/kg	2 to 5

- Liver vacuolation severity score*: none (0), minimal (1), mild (2), moderate (3), marked (4) and severe (5)
- IMS data was collected from selected **low-** and **high-dose** samples and **controls** to evaluate potential differences in compound signals based on *dose* and *vacuolation severity*

Results

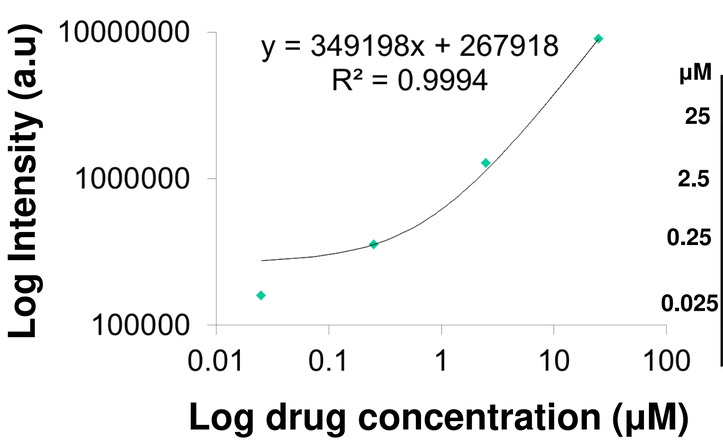
Dose- and severity-dependent increase in the intralesional *parent-drug* signal intensities

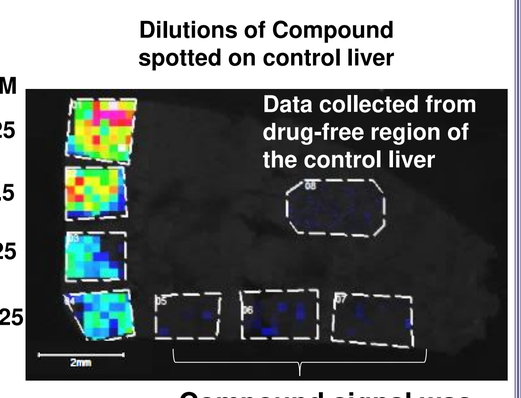


Note:
1. Single sample, 3 sections/sample (*technical*)
2. Multiple samples,1 section/sample (*biological*)
3. One-way ANOVA; mean intensity ± SD
   * P<.05 ** P<.005

Results cont'd

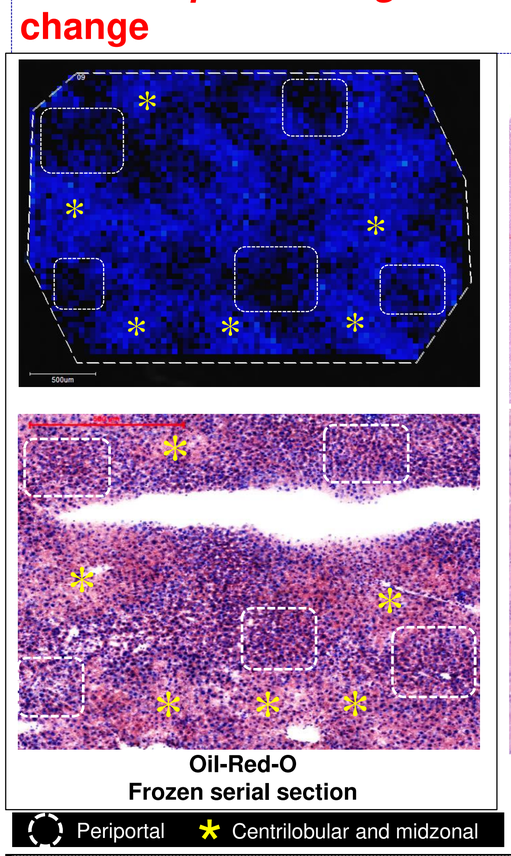
Estimated drug concentration range: 0.1-0.58 µM (Low dose - High dose)

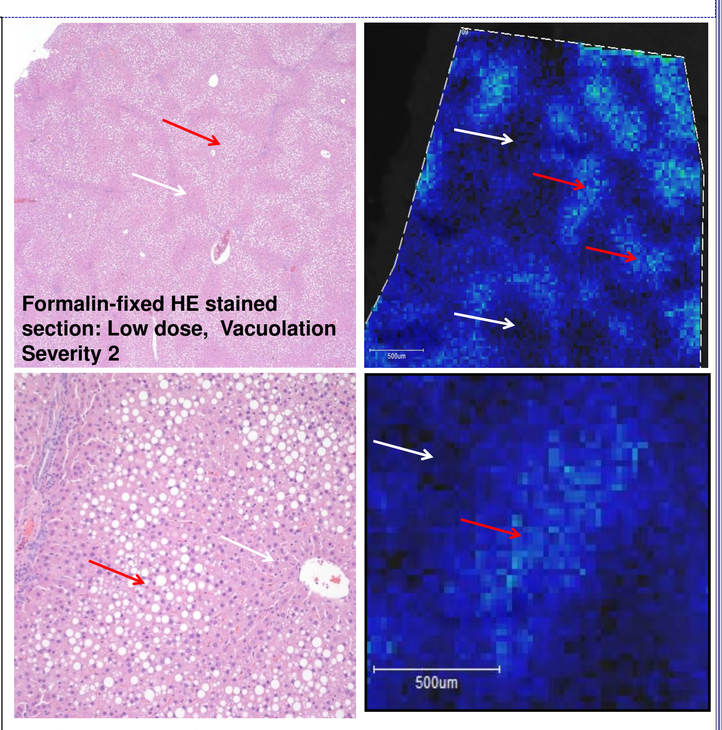




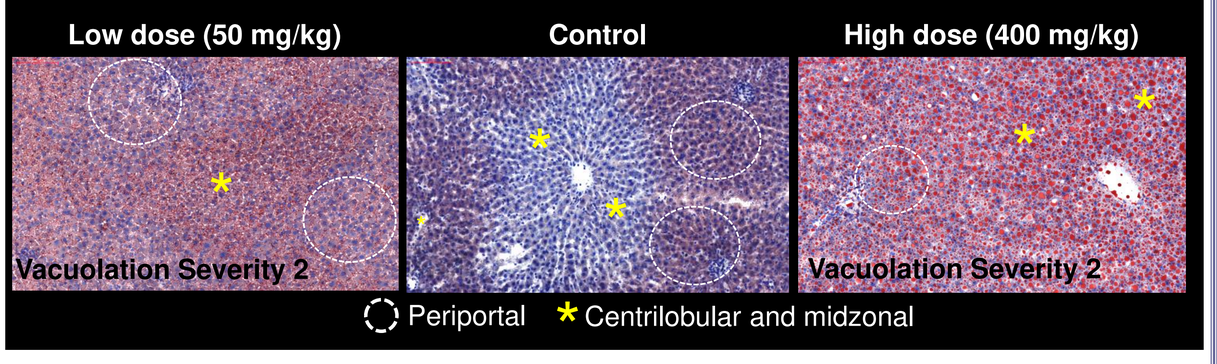
Compound signal was not detected upon further 10-fold dilutions

Co-registration of IMS images with serial sections stained with HE & oil-red-o (lipid histochemical stain) revealed predominantly midzonal *parent-drug* distribution that correlates with vacuolar change



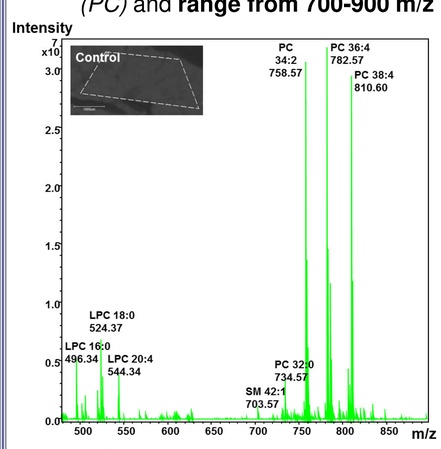


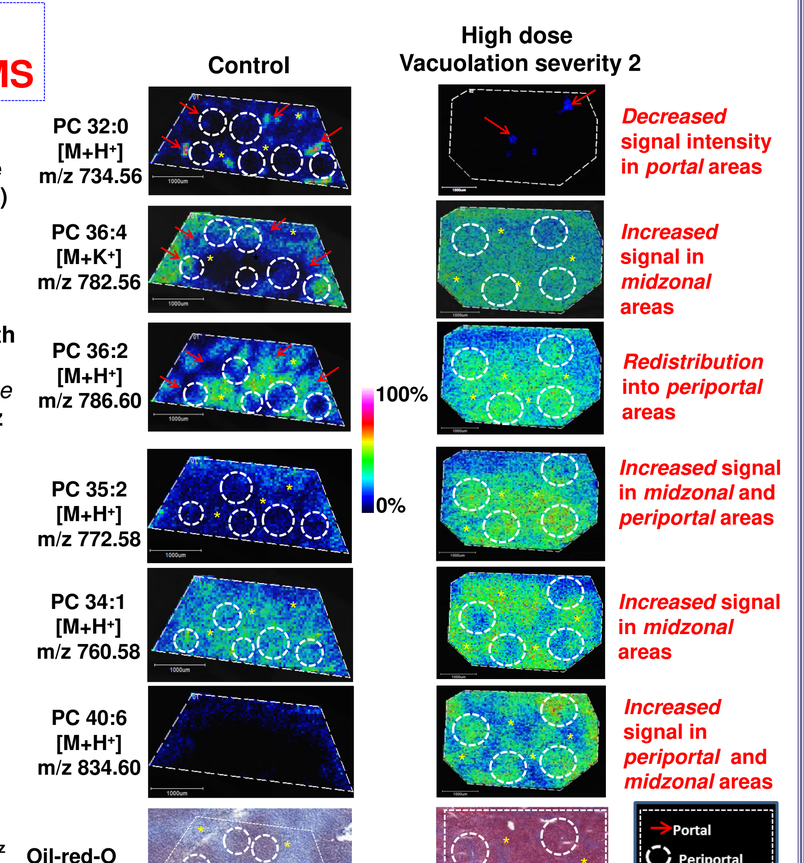
The normal periportal lipid staining pattern observed in control liver appears to have been lost upon drug treatment



Preliminary Lipid Analysis by MALDI-IMS

- **Major lipid classes in liver include: phospholipids (positive charged) > triglycerides (neutral) > cholesterol ester (neutral) > sphingolipids and others** (Christie, WW, J. Lipid Res, 1986)
- **Majority of lipids detected in both *control* and drug-*treated* liver samples were** *Phosphatidylcholine (PC)* and **range from 700-900 m/z**





Conclusions

- MALDI-IMS identified *in situ* parent-drug distribution that correlated with morphologic pattern of hepatic vacuolar change
- Parent-drug localization in vacuolated areas proportional to dose and severity
- Parent-drug signals detected in liver tissue even in the absence of histological evidence of vacuolar change (low dose; vacuolation severity 0)
- No metabolites of parent-drug were detected by MALDI-IMS in liver (*data not shown*)
- Preliminary evaluation of lipids suggests distribution changes in a few *phosphatidylcholine* (PC) species within the hepatic zones in high-dose drug-treated liver samples compared to controls
- Other *neutral* lipid species expected to be enriched in liver such as 'triglycerides and cholesterol esters' were not detected by MALDI-IMS, likely due to their weak ionization
  - Complementary analytical approaches like LC-MS could provide a broad spectrum detection of lipid species
- The findings provide 'proof-of-concept' of the use of MALDI-IMS in Preclinical Safety