Mass spectrometry imaging (MSI) of bile salts and lipids in healthy and diseased liver:

Identification of molecular markers for structural elements of the mammalian liver and their usefulness in MSI of drug-induced liver toxicity

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INTRODUCTION

Matrix-assisted laser desorption ionization-mass spectrometry imaging (MALDI-MSI) is a powerful label-free technique for the simultaneous detection of pharmaceuticals, metabolites and endogenous species in tissue sections, which makes it suitable for toxicological studies.

Aims: To determine the spatial distribution of distinct bile salt species in the liver and to identify lipid molecular markers that define the structural elements of the portal area and liver parenchyma, to improve the evaluation of drug-induced liver toxicity by MSI.

METHODS

MALDI-MSI **(Fig. 1)** was used to monitor the spatial distribution of bile salts and lipids in liver sections of rat, dog and human (normal liver sections or patients with primary sclerosing cholangitis (PSC))

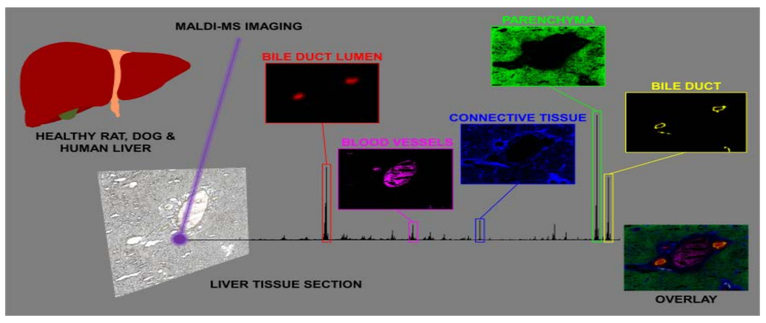
Tissue preparation

- Liver tissues for MSI, were sectioned using a cryo-microtome at a temperature of -20 °C to produce 10 µm-thick sections, which were thaw-mounted onto clean indium tin oxide (ITO)-coated glass slides. Matrix application: Tissue sections were coated with 15 mg/mL DHB in 2:1 $CHCl_3$:MeOH with 0.2% TFA.
- Consecutive 5-µm cryosections were stained with hematoxylin and eosin (H&E) and Masson's trichrome (MTC). The biliary epithelium was stained using a wide spectrum anti-cytokeratin antibody (Dako/Agilent Pathology Solutions).

Instrumentation

- *MALDI-MSI*. High-speed imaging was performed on a Bruker RapifleX MALDI Tissuetyper™ system
- *MALDI-FTICR-MSI.* For confirmation of molecular identity, high-mass resolution (100,000 at *m/z* 500) measurements were performed on a Bruker SolariX FTICR mass spectrometer equipped with a 9.4 T superconducting magnet. The instrument was operated in both positive- and negative-ion modes in the mass range *m/z* 100–2000.
- *MALDI-MS/MS*. For further confirmation of molecular identity, a Waters MALDI HDMS Synapt G2-Si mass spectrometer was used to acquire IMS-MS and tandem mass spectrometry (MS/MS) spectra.

Figure 1. Overview MSI of liver section

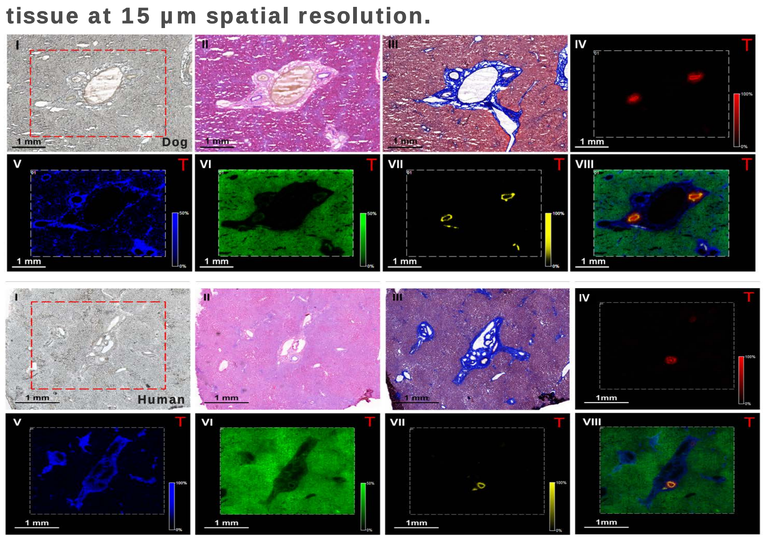


RESULTS

MALDI-MSI in negative ion mode showed the local presence of a variety of bile salts (glycine-, taurine- and unconjugated bile acids), as localized patches of varying sizes, representing the bile ducts, throughout the liver tissue. Specific molecular markers were identified for the connective tissue (a phosphatidic acid [PA (18:0_18:1)–H]$^-$), the liver parenchyma (a phosphatidylinositide [PI (18:0_20:4)-H]$^-$), and the bile ducts (a hydroxyl-sulfatide [ST-OH (18:1_24:0)-H]$^-$) **(Fig. 2)**.

The sulfatide (*m/z* 906.63) was found to be uniquely lining the inside of the bile duct co-localized with cytokeratins, a marker of (bile duct) epithelium, and encased luminal bile salts **(Fig. 4)**.

Figure 2. Multimodal imaging of healthy dog / human liver tissue at 15 µm spatial resolution.



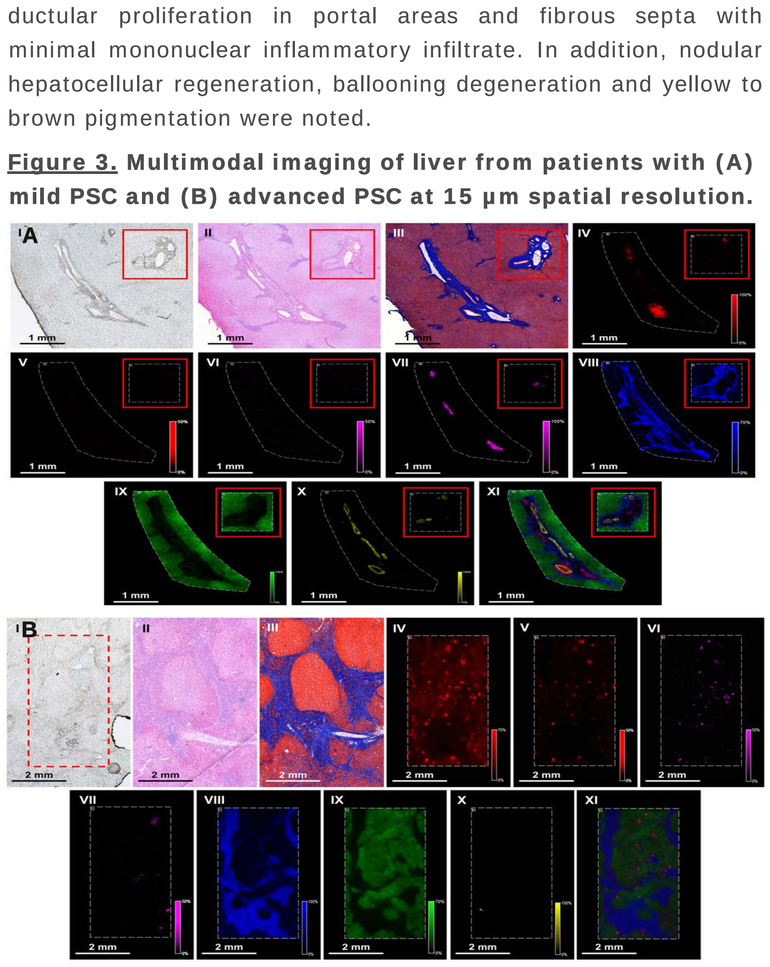
*(I) Optical image, (II) H&E, (III) MTC. MALDI-FTICR-MS images showing the distribution of selected molecular species in the (IV) **bile duct lumen ([TCA-H]$^-$ at m/z 514.28)**; (V) connective tissue ([PA (18:0_18:1)–H]$^-$ at m/z 701.51); (VI) parenchyma ([PI (18:0_20:4)-H]$^-$ at m/z 885.55); (VII) **bile duct ([ST-OH (18:1_24:0)-H]$^-$ at m/z 906.63**) and (VIII) overlay of the selected species.*

PSC patients: The first (mild PSC) had normal liver tests, with no indications for cholestatic liver injury, the second (advanced PSC) had liver test abnormalities indicating cholestasis and liver injury.

Mild PSC. At histology, minimal periductal fibrosis and periductal inflammatory cell infiltrate were noted. Mixed inflammatory cells and bile ductular proliferation were found at the periphery of the portal area extending into the interlobular septa

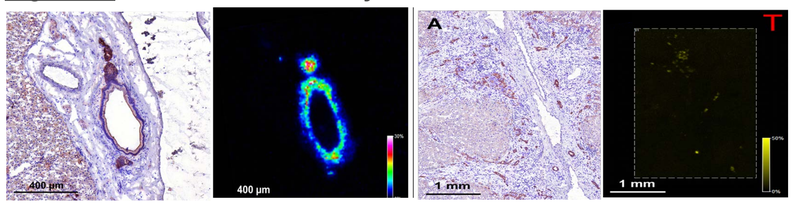
Advanced PSC. Histology showed a diminished number/absence of interlobular bile ducts, portal to portal bridging fibrosis, bile ductular proliferation in portal areas and fibrous septa with minimal mononuclear inflammatory infiltrate. In addition, nodular hepatocellular regeneration, ballooning degeneration and yellow to brown pigmentation were noted.

Figure 3. Multimodal imaging of liver from patients with (A) mild PSC and (B) advanced PSC at 15 µm spatial resolution.



*(I) Optical image, (II) H&E, (III) MTC. (IV-XI) MALDI-FTICR-MS images showing the distribution of selected molecular species. **A**. Mild PSC: Liver tissue showed the same distribution of selected molecular species as healthy human liver tissue. **B**. Advanced PSC: (IV) **Taurine-conjugated bile salts in bile ducts (ductular proliferation) and surrounding parenchyma**. (V) A unique (sulphated) bile salt species at m/z 531.30. (VI) bilirubin diglucuronide ([M-H]$^-$) at m/z 935.32. (VII) heme ([M-H]$^-$) at m/z 615.17. (VIII) lipid at m/z 701.51, [PA (18:0_18:1)-H]$^-$ was abundant in the fibrotic connective tissue (IX) [PI (18:0_20:4)-H]$^-$ at m/z 885.5504. (X) **sulfatides were virtually absent** (considered due to the destruction of large bile ducts)*

Figure 4. Bile ducts in healthy versus advanced PSC liver



Sulfatide (m/z 906.63) co-localized with cytokeratin+ bile duct epithelium versus PSC ductular proliferation (A)

These structural markers were then used to investigate if the toxicology findings of multifocal hepatic necrosis and bile duct hyperplasia with periportal fibrosis and chronic inflammation **(Fig. 5)** observed in the livers of dogs dosed for 14 days with a candidate pharmaceutical compound, could be linked to either the dosed compound or its metabolite(s) with MALDI-MSI.

Figure 5. Bile duct hyperplasia with periportal fibrosis

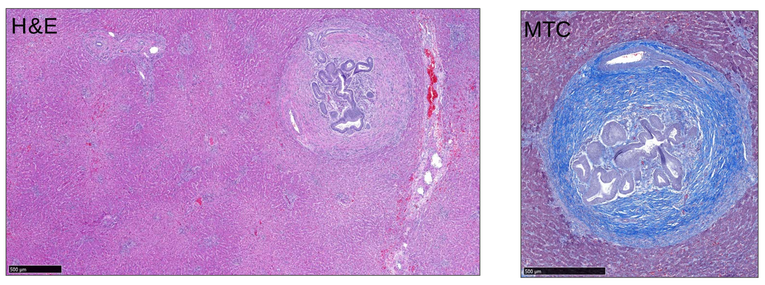
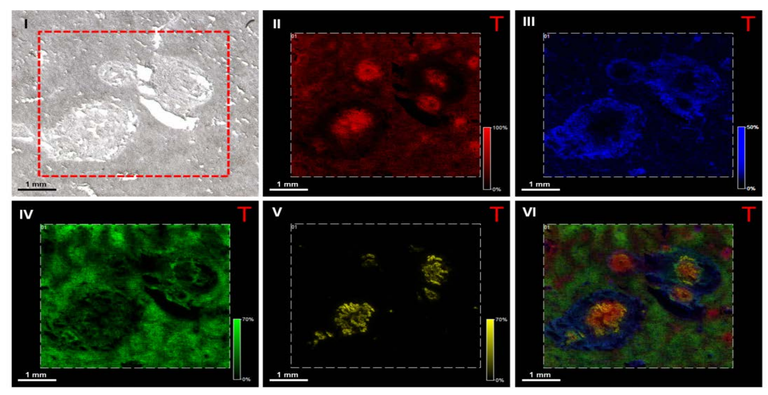


Figure 6. MSI (negative ion mode) of treated dog liver (15 µm)

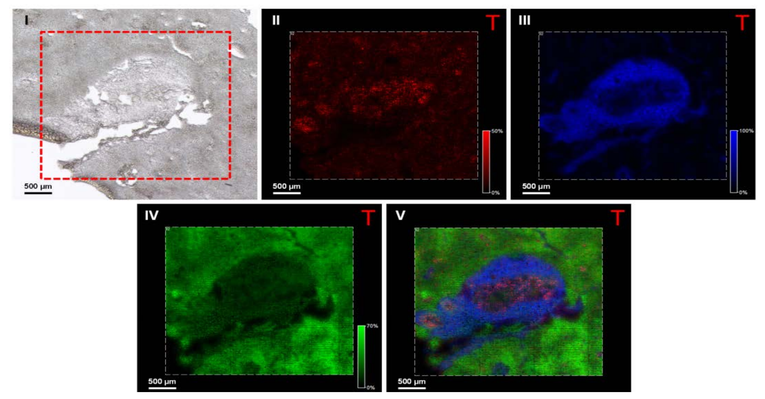


*(I) Optical image, MALDI-FTICR-MS images showing the distribution of (II) **[TCA-H]$^-$ at m/z 514.28,** (III) [SM (18:1_16:0) - CH_3]$^-$ at m/z 687.54, (IV) [PI (18:0_20:4) - H]$^-$ at m/z 885.55, (V) **[ST-OH (18:1_24:0) - H]$^-$ at m/z 906.63** and (VI) overlay of selected species*

The conjugated bile salt, taurocholate (M-H]$^-$) at *m/z* 514.28, was highly localized in the bile duct lumen but also distributed throughout the parenchyma in the affected areas **(Fig. 6)**.

Only the parent compound was detected in the center of the hyperplastic bile ducts surrounded by periportal fibrosis and chronic inflammation **(Fig. 7)**.

Figure 7. MSI (positive ion mode) of treated dog liver (15 µm)



*(I) Optical image, MALDI-FTICR-MS images showing the distribution of (II) **compound** ([M+H]$^+$) at m/z 502. (III) ([PC (32:0)+K]$^+$) at m/z 772.52. defined as a sphingomyelin ([SM (18:1_16:0) + K]$^+$). (IV) ([PC (38:4)+K]$^+$) at m/z 848.54 and (V) overlay of selected species.*

CONCLUSIONS

- Using MALDI-MSI, we identified lipid-specific distributions in different compartments of the liver in all three species (rat, dog, human), in both positive- and negative-ion mode.
- (Hydroxyl)-sulfatides were identified as specific molecular marker for the bile ducts and uniquely localized to bile duct epithelium.
- In the healthy liver specimens, bile salts were confined within the biliary lumen. Upon severe biliary changes they were also observed in the adjacent parenchyma.
- The ability to simultaneously monitor the distribution of drugs and its metabolites, as well as endogenous species, and to correlate this information with standard histological staining is a distinct advantage in pharmaceutical research and toxicological investigations.

This study was conducted by Janssen Research and Development