Mass Spectrometry Imaging in toxicology study: Application in induced interstitial pulmonary fibrosis (IPF) model

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Introduction

The identification of the potential causes of the histopathologic/functional tissue changes is one of the main difficulties in **toxicology** study. Mass spectrometry imaging (**MSI**) technology has been used to address these crucial issues. The main advantage of this **label free** imaging technique is the detection of all molecules of interest directly on-tissues with **high specificity**.

Indeed the molecular distribution of some unlabeled targeted molecules could be directly correlated with some histopathologic and functional tissue changes[1,2]. MSI was used to improve the understanding of the Bleomycin interstitial pulmonary fibrosis rat model. Specially the distribution of lysophosphatidic acid (LPA) was investigated and identify as a potential **therapeutic target** for fibrosis.

Overview

Purpose

- MSI provides high capabilities to localize, identify and quantify drugs and metabolites in complex tissues and allows to follow metabolic pathways as well as identifying new biomarkers or potential readouts.

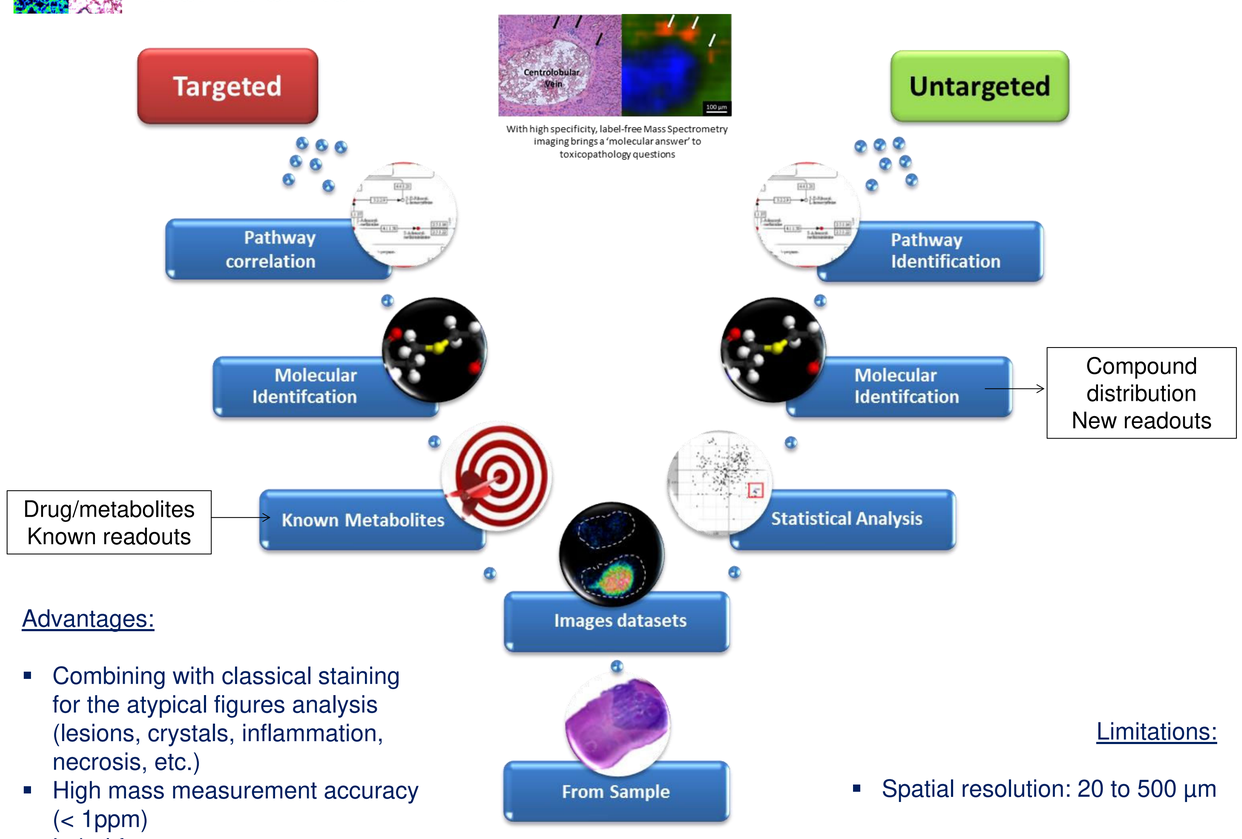
Method

- Combination of histological staining and molecular imaging (MALDI Imaging) without any labeling
- Statistical analysis for the readouts detection
- Structural analysis with a HR Mass Spectrometer (FTICR)

Results

- Identification of potential readouts /markers of the model
- No detection of the Bleomycin by MSI in the treated tissues

MSI worflow



Advantages:

- Combining with classical staining for the atypical figures analysis (lesions, crystals, inflammation, necrosis, etc.)
- High mass measurement accuracy (< 1ppm)
- Label free
- Quantitative

Limitations:

- Spatial resolution: 20 to 500 µm

Figure 2: *General workflow of the MSI approach in tox studies*

Readout distribution in IPF tissue model

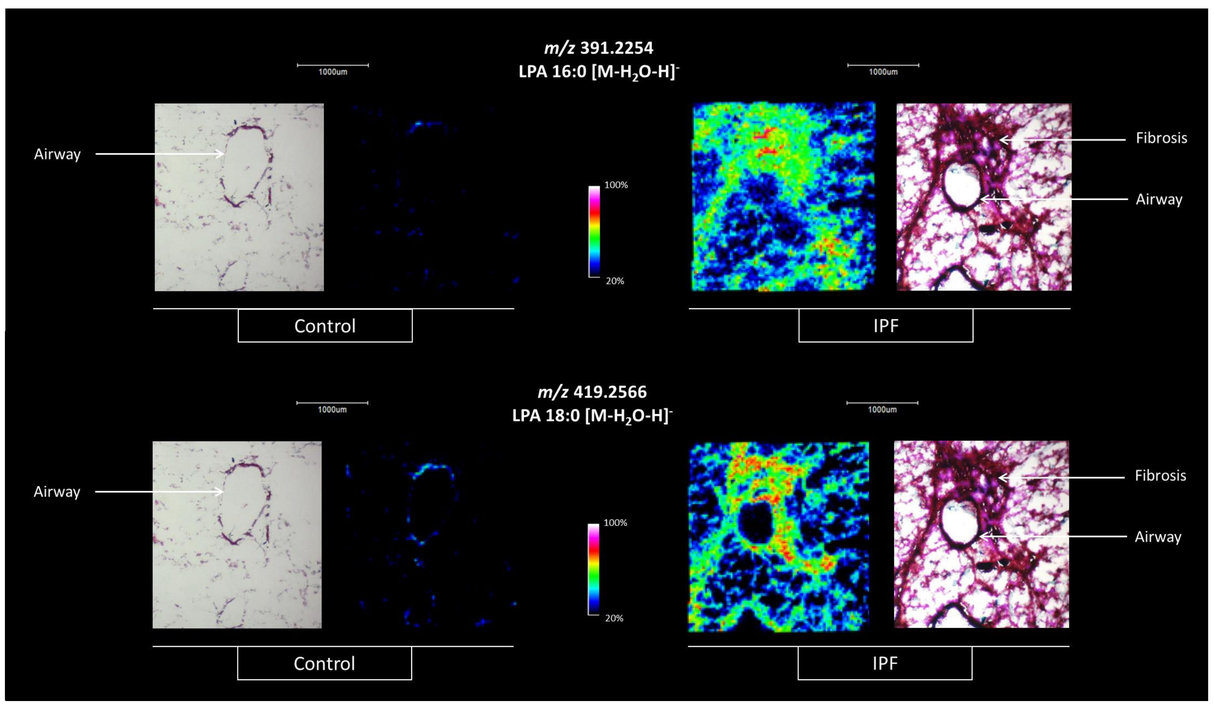


Figure 3: *LPA 18:0 and LPA 16:0 molecular distributions comparison between the control and IPF tissues by MSI*

After the specific marker identification (table 1), the molecular distributions were used to confirm the difference between the control and the Bleomycin groups (figure 3). As visualized, a significative difference of signal was observed for both markers in the IPF tissues compared to the control: LPAs are clearly distributed in the fibrosis area.

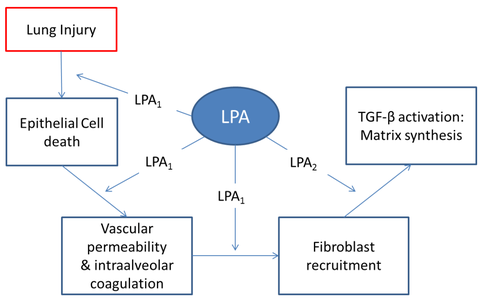


Figure 4: *Biological processes implicated in lung fibrosis and regulated by LPA*

LPA is described in the literature[3,4] to contribute to the development of fibrosis after lung injury through multiple mechanisms (via LPA1 and LPA2 receptors), including the induction of (figure 4):

1. epithelial cell apoptosis
2. increased vascular permeability, resulting in increased intra-alveolar coagulation
3. fibroblast recruitment into the injured airspaces and their resistance to apoptosis
4. activation of latent TGF-b

Experimental

Sample preparation

Rats were administered seven doses of bleomycin delivered to lung by the oropharangeal aspiration route and followed for 22 days. Control animals received seven doses of saline. Three control and three treated animals were sacrificed and lungs were collected. An agarose inflation was performed to conserve the histological structures during the cryosection process.

Several fresh sections were prepared using cryostat CM3050S (Leica, Germany) at -35°C and mounted on ITO conductive glass slides.

Molecular Imaging

2.5 DHB matrix (40mg/ml Methanol/TFA 0.1% 1:1; v:v) or 9AA matrix (5mg/ml Methanol 80%) were used by spraying it with the SunCollect system (Sunchrom GmbH, Friedrichsdorf, Germany).

A MALDI-FTICR (7T Solarix, Bruker Daltonics, Bremen, Germany) with SmartBeam II laser was used in positive (DHB matrix) or negative (9AA matrix) with a fullscan mode of acquisition within 100-1000 Da mass range at 30 µm spatial resolution (figure 1).

Figure 1: *HR-MSI platform*

Masson's trichrome staining

A Masson's trichrome staining (light green for collagen) was performed on each fresh tissue section after the MALDI imaging acquisition for combining and comparing the staining with the molecular distribution.

Data analysis and image construction

ImaData bank: unique databank of molecules detected by Mass Spectrometry Imaging and MALDI Imaging in every tissues. 2 500 molecules have been identified, organized in a databank and associated with a unique statistical tools to interpret imaging experiments.
Quantinetix 1.7.1 and Fleximaging 4.0 were used for the image construction and normalization.
ImaStation 1.0 was used to perform the statistical analysis.

Untargeted IPF readout identification (100 - 1,000 Da)

An **untargeted** approach was used to determine and identify some potential readouts/markers of the fibrosis between 100 to 1,000 Da:

1. Statistical comparison of the bleomycin and control group (T-test with unequal variances) and average intensities based on MSI data set (more than 20,000 spectra per image)
2. Differential list analysis: potential markers of the IPF selection based on the foldchange compared to the control (complete areas)
3. Identification of the compound based on the mass accuracy (<1ppm) and on ImaData bank (metabolites) interrogation (results example for the LPA in table 1)
4. Confirmation of the readout specificity based on the molecular distribution comparison (figure 3)

Name	Ionization mode	Ion form	*m/z* exp	*m/z* theo	|Error| (ppm)	IPF Average signal	Control average signal	p-value	Foldchange (total area IPF/control)
LPA 16:0	negative	$[M-H_2O-H]^-$	391.2254	391.2255	0.4	639235	357774	<0.0001	2
LPA 16:0	negative	$[M-H]^-$	409.2359	409.2361	0.4	186972	109780	<0.0001	2
LPA 18:1	negative	$[M-H_2O-H]^-$	417.2409	417.2411	0.6	333998	182572	<0.0001	2
LPA 18:0	negative	$[M-H_2O-H]^-$	419.2566	419.2568	0.5	1105291	638364	<0.0001	2
LPA 18:0	negative	$[M-H]^-$	437.2673	437.2674	0.2	302860	171921	<0.0001	2
...	...	...	...	...	...	...	...	...	...

Table 1: *Example of readout identification by MSI after statistical comparison (T-test with unequal variances) between IPF and control tissues*

Conclusions

- ✓ Based on the combination of **MSI** and staining, LPA were detected and confirmed to be **specifically distributed** in the fibrosis.
- ✓ In this study, the bleomycin was not detected by MSI in the fibrosis area of the treated tissues
- ✓ Some other **specific ions** of the fibrosis are being identified
- ✓ The analysis could be performed for a higher mass range (>1,000 Da)
- ✓ MSI is a powerful tool for the **makers/readout** analysis in **toxicology** studies associated to atypical figures or biological processes

Literature

1. Castellino, S., M. R. Groseclose, et al. (2012). Chemical Research in Toxicology, American Chemical Society.
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4. Shea, B. S. and A. M. Tager (2012). Proc Am Thorac Soc 9(3): 102-10.