M0930-06-32

Development of Quantitative MALDI Mass Spectrometry Imaging methods for studying distribution of antituberculosis drugs at their site of action

Mathieu Gaudin, Elisa Hivin, Guillaume Hochart, Corinne Ramos, David Bonnel

Aliri France SAS, 152 Rue du Dr Yersin, 59120 Loos, France

info@aliribio.com

PURPOSE

Tuberculosis (TB), caused by *Mycobacterium tuberculosis* (MTb), remains a global health challenge, with treatment involving a year-long regimen of four drugs: Isoniazid, Rifampicin, Pyrazinamide, and Ethambutol. However, the emergence of drug resistance calls for the development of new therapeutic molecules. Due to the complex granulomatous lesions formed by MTb, plasma drug concentrations often do not reflect tissue drug levels, making it crucial to assess drug exposure at the site of action. To address this, we have developed Mass Spectrometry Imaging (MSI) methods to study the distribution of anti-TB drugs and their metabolites, including pyrazinoic acid, across both healthy and diseased tissues.

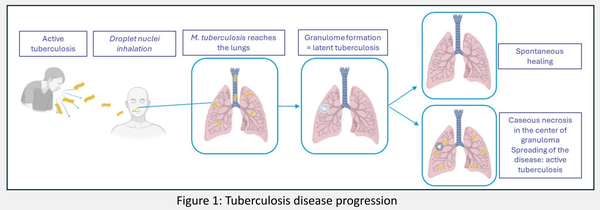

Figure 1: Tuberculosis disease progression

METHOD(S)

Untreated frozen rat lungs were cryosectioned using a cryostat and mounted on Indium-Tin-Oxide glass slides (Delta Tech, Loveland, CO, USA). Dilution series of each drug were prepared and spotted on the control tissues before coating the slide with various MALDI matrices, solvents, and derivatization agents when necessary, using an automated sprayer (TM sprayer, HTX Imaging, Chapel Hill, NC, USA).

Slides were imaged using a MALDI FTICR (Solarix 2XR, Bruker, Bremen, Germany) using QuadruPolar Detection to maximize spectral resolution and method specificity. Calibration curves were obtained using Multimaging 1.3.2.1 software (Aliri, Loos, France).

Non-infected non-human primates (NHP) lungs treated with Pyrazinamide were sectioned at 10 µm thickness, mice treated with 100 mg/kg Ethambutol (p.o.) were sectioned at 20 µm. Further slide preparation was performed according to the previous method development.

RESULTS

- Limit of detection (LOD), Lower Limit of Quantification (LLOQ) and Upper Limit of Quantification (ULOQ) for the different antituberculosis drugs with slide preparation information are summarized in Table 1.
Most of the compounds led to LLOQ in the low µg/g range.

- Due to the low molecular weight and high polarity of **Isoniazid**, optimized on-tissue chemical derivatization with trans-cinnamaldehyde (CA) adapted from Manier *et al* [1] was performed. CA was sprayed on tissue instead of using CA pre-coated slides to which an extra washing step of the tissue section was added, to remove hydrophobic interfering species and decrease ion suppression to eventually obtain a sensitivity of 1.29 µg/g, compatible with expected tissue exposure at therapeutic doses. MALDI MSI calibration curve of isoniazid is presented in Figure 2 and shows good linearity.

- Due to the low sensitivity of **pyrazinamide** detection, an alternative method was developed by derivatizing its active metabolite, pyrazinoic acid, using the CAX-B agent (Figure 3). While pyrazinoic acid alone does not represent the full drug exposure, as it is the active form generated by *Mycobacterium tuberculosis* pyrazidamidase, this method provides insights into its action within granulomatous lesions. Unexpectedly, MALDI-FTICR analysis revealed pyrazinoic acid in NHP lungs, suggesting that host hydrolytic enzymes might also produce this metabolite, beyond bacterial activity (Calibration curve in Figure 2 and MSI in Figure 4).

- QMSI revealed very high concentrations of **ethambutol** at T4h in the stomach and intestines (>ULOQ) and moderate concentrations in other organs such as the kidney, liver, pancreas or lung (30-60 µg/g of tissue). No detection of the drug was observed in the brain, muscles or myocardium (Figure 5).

Molecules	Washing	Derivatization	Matrix	Composition	LOD (µg/g)	LLOQ (µg/g)	ULOQ (µg/g)
Isoniazide	Yes	CA	DHB 40 mg/mL	1:1 MeOH:Water +0.1% TFA	1.29	1.29	801
Rifampicine	No	None	DAN 10 mg/mL	MeOH:H2O 50:50	2.52	2.52	1162
Pyrazinamide	No	None	DHB 40 mg/mL	1:1 MeOH:Water +0.1% TFA	81.4	81.4	269
Pyrazinoic acid	No	CAX-B	DHB 40 mg/mL	1:1 MeOH:Water +0.1% TFA	1.76	7.76	378
Ethambutol	Yes	None	DHB 40 mg/mL	1:1 MeOH:Water +0.1% TFA	1.55	1.55	652
Bedaquilin	No	None	DHB 40 mg/mL	1:1 MeOH:Water +0.1% TFA	4.31	4.31	70.5
Pretomanid	Yes	None	DHB 40 mg/mL	1:1 MeOH:Water +0.1% TFA	2.51	2.51	403
Moxifloxacin	Yes	None	DHB 40 mg/mL	1:1 MeOH:Water +0.1% TFA	2.77	2.77	287
Rifapentin	No	None	9AA 10 mg/mL	90/10 IPA/H2O	3.56	5.3	300

Table 1: LOD, LLOQ and ULOQ of the antituberculosis drugs on tissue

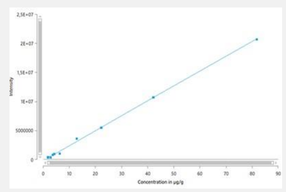

Figure 2: Calibration curve of derivatized Isoniazid

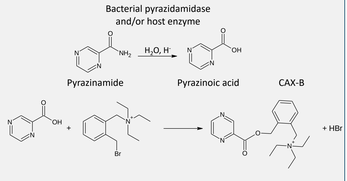

Figure 3: Derivatization of pyrazinoic acid with CAX-B

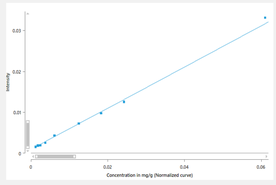

Figure 3: Calibration curve of derivatized Pyrazinoic acid

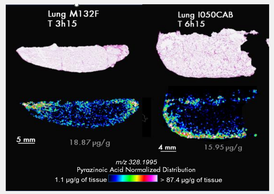

Figure 4: MSI and QMSI of pyrazinoic acid in NHP lungs (Top: H&E staining after imaging; bottom: MSI only)

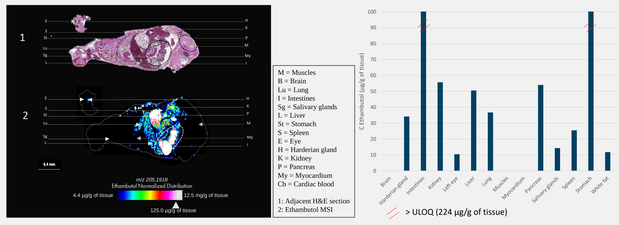

Figure 5: MSI and QMSI of Ethambutol in mouse whole-body section (T4h)

CONCLUSION(S)

A complete set of MALDI QMSI methods has been developed to serve the need of the consortium ERA4TB to study the distribution of multiple drugs to be tested either alone or in combination regimens in multiple preclinical models of infected or non-infected animals.

This platform offers opportunities for studying pharmacokinetics at the site of action, refining PBPK models, studying mechanism of action, drug-drug interactions, or host-pathogen interactions under treatment.

REFERENCE(S)

[1] Manier *et al*. Reagent Precoated Targets for Rapid In-Tissue Derivatization of the Anti-Tuberculosis Drug Isoniazid Followed by MALDI Imaging Mass Spectrometry. *J. Am. Soc. Mass Spectrom*. (2011) 22:1409-1419.

FUNDING

Funding from the Innovative Medicines Initiative 2 Joint Undertaking (JU) under grant agreement No 853989. The JU receives support from the European Union's Horizon 2020 Research and Innovation Programme and EFPIA and Global Alliance for TB Drug Development Non-Profit Organization, Bill & Melinda Gates Foundation, University of Dundee. This work reflects only the author's views, and the JU is not responsible for any use that may be made of the information it contains.