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Distribution of standard-of-care antituberculosis drugs in cynomolgus macaque lungs

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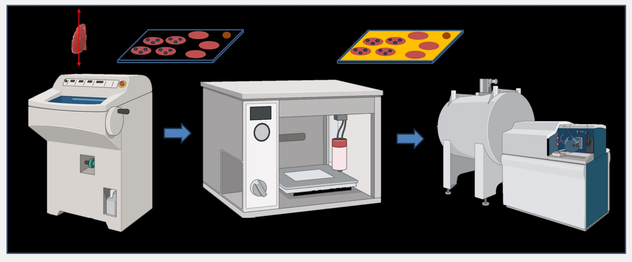
PURPOSE

The non-human primate (NHP) provides the most clinically relevant model of human tuberculosis. The aim of this study was to determine the tissue pharmacokinetics of orally delivered isoniazid (H), rifampicin (R), pyrazinamide (Z) and ethambutol (E) combination therapy (HRZE) in the lung of cynomolgus macaques to prepare following studies in Mycobacterium tuberculosis-infected animals usinq QMSI.

METHOD(S)

Six cynomolgus macaques received a single oral delivery of isoniazid at 15 mg/kg, rifampicin at 15 mg/kg, pyrazinamide at 200 mg/kg and ethambutol at 75 mg/kg. Necropsies were conducted 2h45, 3h15, 3h30, 5h30, 6h15, 24h after drug administration at which the upper right lung lobe was collected, inflated with agarose, snap frozen and shipped to Aliri.
Lung samples were cryosectioned using a cryostat and mounted on Indium-Tin-Oxide glass slides (Delta Tech, Loveland, CO, USA). Dilution series of each of the 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 MALDI FTICR mass spectrometers (SolariX and Solarix 2XR, Bruker, Bremen, Germany). Calibration curves were obtained using Multimaging 1.3.2.1 software (Aliri, Loos, France) (workflow in Figure 1). Adjacent sections collected on Superfrost slides were stained with Hematoxylin-Eosin to produce histological images which have been overlayed with the MSI datasets.

Figure 1: General workflow for MSI



RESULT(S)

- Despite the use of a specific derivatization protocol with a limit of detection of around 2 µg/g of tissue, **isoniazid** was barely detected in the samples investigated.

- Conversely, QMSI revealed a wide distribution of **ethambutol** up to 24h after administration of around 30 µg/g (Figure 2).

- As the pyrazinamide limit of detection was too high to enable imaging at therapeutic doses, its active metabolite **pyrazinoic acid** was imaged as a surrogate, pending further analytical development on the parent drug. Pyrazinoic acid was detected in lung tissue collected at each of the timepoints investigated (Figure 3), the concentration-time profile was built (Figure 4) and PK parameters obtained (Table 1).

Although pyrazinoic acid was thought to be produced in the granulomatous lesions by bacterial pyrazidamidase activity, MALDI-FTICR MSI revealed the presence of pyrazinoic acid in non-infected NHP, suggesting an additional production of this metabolite by a hydrolytic enzyme of the host.

Figure 2: Spatial distribution of Ethambutol in NHP Lungs up to T24h

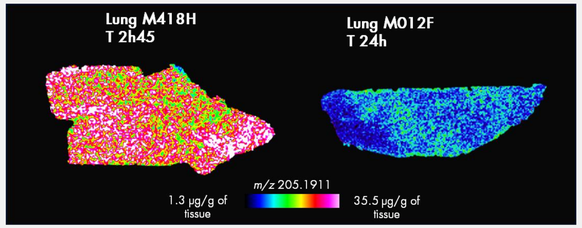


Figure 3: Spatial distribution of Pyrazinoic acid in NHP Lungs including H&E staining sections and MSI sections.

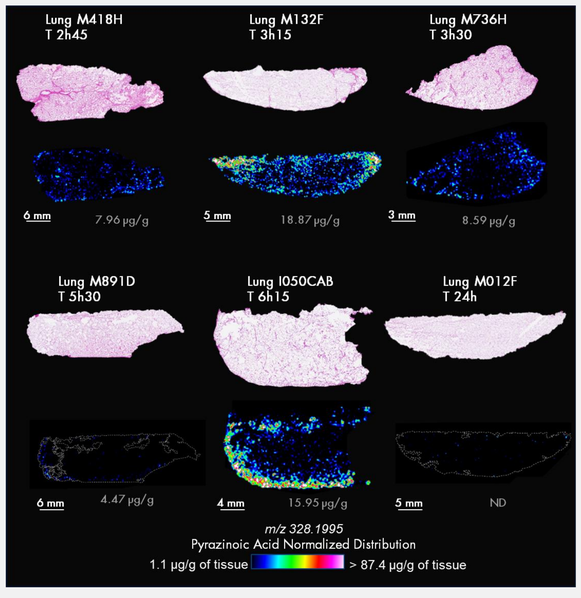


Figure 4: PK profile of Pyrazinoic acid in NHP Lungs

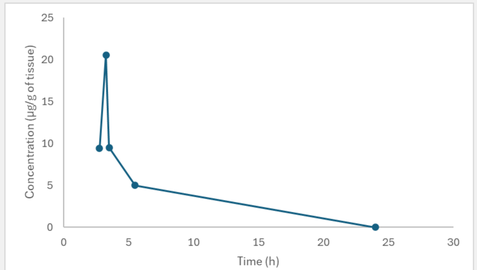


Table 1: PK parameters of Pyrazinoic acid in NHP Lungs

Parameter	Unit	Value
Lambda_z	1/h	0.1306
t1/2	h	5.31
Tmax	h	3.25
Cmax	µg/g	18.87
AUC 0-24	µg/g*h	80.12
AUC 0-inf_obs	µg/g*h	83.95
AUC 0-24/0-inf_obs		95%

CONCLUSION(S)

While the study was limited to evaluating a single animal at each time point for obvious ethical reasons, it highlighted the potential for variation in response to drug treatment. Some animals (e.g., animal ID I050CAB, T 6h15) exhibited very different ADME behavior toward HRZE combination therapy, with much higher exposure than expected from the concentration-time profile built with the other animals.

This consideration should be taken into account when building PBPK models of antituberculosis drugs with a limited number of NHP. This study produced reference HRZE distribution data in the cynomolgus macaque lung and contributes to a better understanding of the PK of standard-of-care antituberculosis drugs, which will assist in optimizing dosing regimens in anti-TB combination therapies.

From a methodological perspective, this imaging experiment also paved the way for future determination of drug concentration in lesions (granuloma, inner and outer caseum, etc.) in infected cynomolgus macaques to support PK/PD investigations.

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