Title (20 words)

Large scale, multi-instrument MALDI-MSI study into lipidosis in inhalation dosed rats

Authors

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Introduction (120 words max)

Matrix assisted laser desorption ionisation mass spectrometry imaging (MALDI-MSI) is increasingly being used to provide spatially resolved information on the molecular composition of a wide variety of drug treated and pathologically affected tissues. One of the most commonly detected molecular classes by MALDI MSI are phospholipids. Within drug development and pre-clinical trials a common, significant and poorly understood barrier to success is the development of phospholipidosis in tissues and cells as a result of the drug treatment. Significant among this pathology, within inhaled medicine, is the development of foamy macrophages (FM) and fibrosis within the lung. In order to assess lung phenotypes as a result of inhaled drug and nanoparticle treatments MALDI-MSI was employed alongside large scale cell screening methods.

(120 words)

Methods (120 words max)

Murine lungs stored as either fresh frozen of formalin fixed then frozen and were sectioned at 16 µm thickness. Wistar Han rats were treated with either Saline, Amiodarone or PVAc nanoparticles by intranasal administration. Six sections (three serial from two locations; upper lung: across superior lobe near bronchial entrance and lower lung: across inferior and middle lobes) were acquired per lung (performed in triplicate, 18 sections per lung). CHCA (5 mg/mL, 80% CH_3OH, 0.1% TFA) was deposited by TM Sprayer. Data were acquired with a Synapt G2-Si with 2.5 kHz Nd:YAG (Waters) or a QSTAR QqToF (Sciex) with Nd:YAG laser (Elforlight). Images were acquired in either higher or lower throughput modes using pixel sizes of 200 µm or 50 µm. (word count = 120)

Preliminary Data (300 words max)

Within phase one of this project MALDI-MSI was used to acquire data from murine lungs stored in either fresh frozen of formalin fixed then frozen forms. Lungs from inhalation dosed rats (un-dosed, Amiodarone or PVAc nanoparticle; n=1) were also analysed to provide proof of concept data for a larger scale drug inhalation study. Good quality data were obtained from both sample preservation methods. Multivariate analysis (MVA) methods including principal component analysis (PCA) and non-negative matrix factorisation (NMF) were employed for data reduction to aid interpretation. Factors within both the PCA and NMF showed differential lipid profiles within the nanoparticle dosed lung section.

Phase two has begun with a larger scale study into the aforementioned lipidosis phenomena resulting from inhalation dosing of various drugs and nanoparticles. Tissue sections were taken in the triplicate manor described above, allowing three versions of this study to be performed in the following way: lower throughput (~ 6 hours per section), higher spatial resolution (50 µm), higher mass resolving power (~20 – 60,000) and lower limit-of-detection (> 100 times that of the QSTAR) data was acquired on the Synapt G2-Si and compared to high (10 – 30 minutes per section) or moderate (2 hours) throughput, lower (200 µm) or higher (50 µm) spatial resolution, low mass resolving power (10,000) data on the QSTAR.

A major aim of this study is to better understand the pixel size, mass resolution, limits-of-detection and consequent analysis time requirements for large scale MALDI-MSI studies of this nature through the acquisition of both low and high throughput, mass and spatial resolution data.

Ongoing aims within this project relate to the assessment of MALDI-MSI as a tool to assess lipidosis and related pathologies alongside cell screening methods providing information issues such as cell morphometrics, cell counting and phagocytosis.

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Novel Aspects (20 words max)

Large scale, multi-instrument MALDI-MSI study into lipidosis in the lungs of inhalation dosed rats

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