MALDI IMS identifies changes in lipids and metabolites in rat brains following arsenic exposure.

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Abstract

Matrix assisted laser desorption ionization imaging mass spectrometry (MALDI IMS) is a label free, ever-evolving technology, which produces 2D ion density maps representing the distribution of an analyte(s) across a tissue section. Correlation of MALDI images with H&E or immunohistochemistry (IHC) stained serial sections provides a "molecular map" to assess an analyte's location and relative intensity to specific cell types and/or tissue architecture. Although this mass spectrometry (MS)-based approach was initially developed to analyze larger analytes such as proteins and peptides, the recent incorporation of high-resolution instruments, such as the Fourier- transform ion cyclotron resonance (FTICR) mass spectrometer within imaging workflows has increased the number of detectable analytes, making identification of lipids, n-linked glycans and small molecule metabolites feasible. MALDI IMS use across toxicity studies has been on the rise, specifically in neurotoxicology studies targeting specific brain abnormalities or changes due to exposure to toxic materials or drugs. A recent study at NCTR was conducted to determine the effects of exposure to inorganic arsenic on development, MALDI IMS was utilized to gain a more thorough understanding of potential toxicity effects on the brain. Brain tissue from postnatal day 21 and adult Sprague-Dawley rats were analyzed, with the adult animals representing long-term effects. Four arsenic treatment groups were tested: 0, 0.10, 1.50, and 3.75 mg/kg/day, with a maximum of 3-4/sex/treatment group. Dose-dependent changes and distributions at specific brain regions were assessed for markers of toxicity in relation to histopathology. Interestingly, MALDI IMS identified many lipids which have been linked to arsenic exposure in mice, phosphatidylcholines (PCs) of varying chain lengths-: PC (36:1), PC (40:5), and PC (38:6). These studies are ongoing, but the preliminary data showing distribution of lipids and metabolites in relation to arsenic exposure will be presented.

Introduction

In 2016, the FDA issued a risk assessment report detailing a major concern regarding fetal susceptibility to neurotoxic effects from maternal dietary arsenic exposure. To address these knowledge gaps, a study using a Sprague Dawley rat model was conducted to assess neurobehavioral and neurochemical effects from exposure to varying doses of arsenite during pre- and post-natal developmental periods. was utilized to assess non-standard neurotoxic endpoints including lipids and metabolites across the arsenic-exposed brain tissue sections from juvenile and adult rats.

The views and opinions expressed are those of the authors and do not necessarily reflect those of the FDA or U.S. Government.

Materials and Methods

Fresh frozen rat brains collected at postnatal day (PND) 21 and 180 (juvenile and adult, respectively) were serial sectioned at 12 µm (coronal and sagittal) for MALDI IMS and H&E, cresyl violet or lipid staining. Tissue sections for MALDI IMS analysis were sprayed with 2,5-dihydroxybenzoic acid (DHB) matrix (40mg/ml). Lipids and metabolites were detected using a Bruker 7T ScimaX MRMS in broadband mode for both positive and negative ion modes. Raster width varied (20-100 µm) and was scan dependent. Lipids and metabolites were identified by accurate mass and confirmed using collision-induced dissociation (CID). MALDI images were acquired using Flex Imaging. Flex Analysis and SCiLS lab were used for processing and alignment of images to stained serial sections

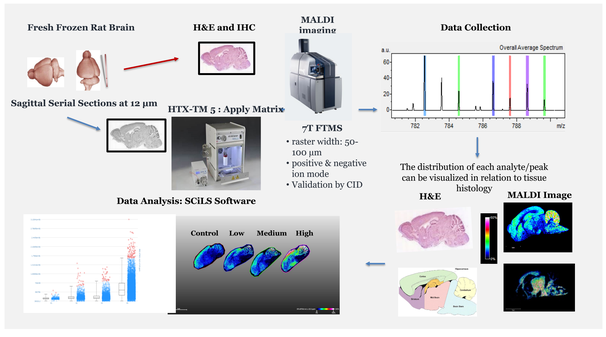

Figure 1. Overview of MALDI IMS method. Fresh frozen brains were sectioned at 12 µm, sprayed with DHB matrix and analyzed via 7T FTICR MS. Each peak (m/z) represents an analyte and it's distribution can be visualized across the brain tissue.

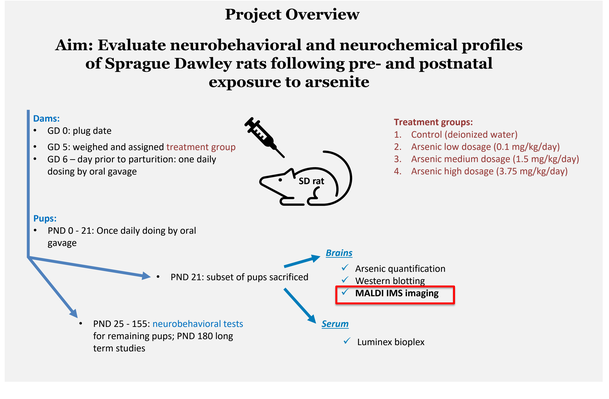

Figure 2. Project Overview. Sprague Dawley rats PND 0-21 were supplied once daily dosing by oral gavage (see treatment groups) and sacrificed for assessment at various endpoints. PND 25-155 and PND180 were assessed for neurobehavioral studies. MALDI IMS analysis was utilized for rat brains from each treatment group.

Results and Discussion

- Flanigan et al. (2021) reported that brain arsenic levels reflected the arensite dosage groups, but that there were no differences noted in neurobehavior and no significant changes in biological markers of neurotoxicity, highlighted limitations for modeling arsenic brain exposures between different species.

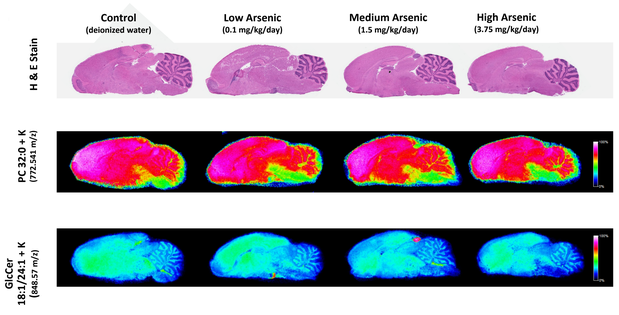

Figure 3. Distribution of two abundant lipid species across the treatment groups. Brain sections were analyze by MALDI IMS to identify lipid species of interest. Lipid distributions could then be compared across the four treatment groups. Many abundant lipids had similar distributions across the four groups, including PC 32:0 +K and GlcCer 18:1/24:1+K.

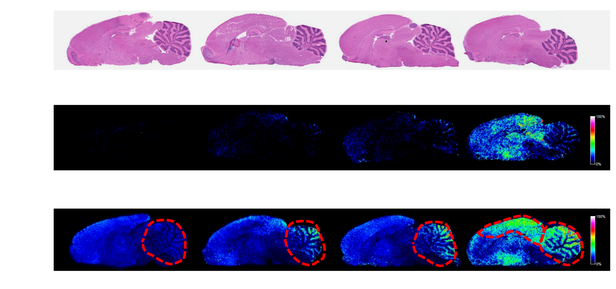

Figure 4. Distribution of two lipid species with increased distribution across the treatment groups. Brain sections were analyze by MALDI IMS to identify lipid species of interest. Lipid distributions were then compared across the four treatment groups. These two lipids exhibited dose-dependent increases in regions throughout the brain. Specific areas of the brain are highlighted.

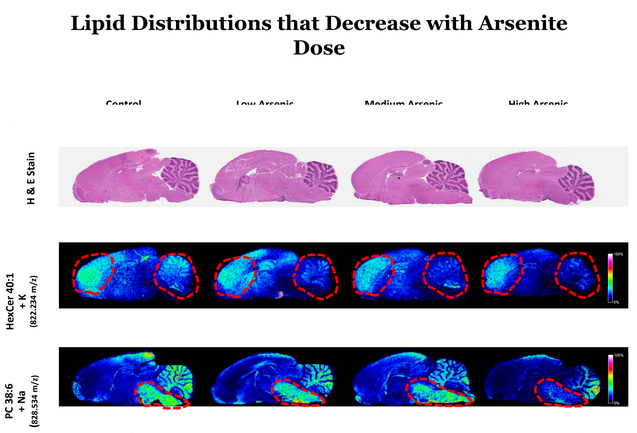

Figure 5. Distribution of two lipid species with increased distribution across the treatment groups. Brain sections were analyzed by MALDI IMS to identify lipid species of interest. Lipid distributions were then compared across the four treatment groups. These two lipids exhibited dose-dependent decreases in several regions throughout the brain. Specific areas of the brain are highlighted.

Conclusions and Future Directions

- **Flanigan et. al. did not identify any differences in neurobehavior or biological markers of neurotoxicity with Arsenic exposures in a Sprague Dawley rats**
- **MALDI IMS analysis is an innovative approach to assess analyte distributions in brain tissues dosed with arsenic**
- **Lipids with differential distributions can be identified and mapped to specific areas in relation to tissue pathology and arsenic exposure.**
- **All identified analytes will be verified using collision induced dissociation.**

References

1. Flanigan TJ, Ferguson SA, Law CD, Rosas-Hernandez H, Cuevas-Martinez E, Fitzpatrick S, Shen AN. Neurobehavioral and neurochemical effects of perinatal arsenite exposure in Sprague-Dawley rats. Neurotoxicol Teratol. 2022 Mar-Apr;90:107059. doi: 10.1016/j.ntt.2021.107059. Epub 2021 Dec 31. PMID: 34979254.