MALDI imaging mass spectrometry of mouse fetuses to assess markers of neural tube defects after maternal opioid exposure

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Abstract

In 2015, FDA released a Drug Safety Communication regarding a possible link between opioid exposure during early pregnancy and an increased risk of neural tube defects (NTDs). At the time, FDA did not make new recommendations for opioid use during pregnancy due to incomplete maternal toxicity data and limitations in both human and animal studies. Since then, FDA scientists have conducted multiple comprehensive studies designed to determine whether the administration of opioids induces NTDs in association with opioid-induced maternal toxicity. In this study, lipid changes in mouse fetuses following exposure to methadone, morphine, or the positive control valproic acid (VPA) were evaluated using matrix assisted laser desorption ionization imaging mass spectrometry (MALDI IMS). MALDI IMS is a mass spectrometry-based approach that provides the distribution and localization of an analyte(s) of interest across an organ or whole body tissue section. Following maternal exposure to the drugs, whole body mouse fetuses with and without treatment related NTDs were analyzed using MALDI IMS. Differences in glycerophospholipids and other lipids could be identified in fetuses related to opioid dose and exposure and presence or absence of NTDs. The differential lipid distributions identified across the fetal tissues included several phosphatidylcholine (PC) species including PC 34:2, PC 34:1, and PC 32:1, which were altered in sagittal and coronal sections and are associated with maternal hypoxia. Additionally, changes in the distribution of the inflammation lipid marker lysoPC 16:0 was observed in the brain region. Lipid identities were confirmed with collision-induced dissociation (CID) for analyte fragmentation. MALDI images were also aligned to H&E and immunohistochemistry (IHC) stained serial sections to map these distributions to histopathology. These findings represent the first MALDI IMS study of whole-body fetuses with opioid exposure during pregnancy. The observed changes in lipid distribution within fetal neural tissues and drug-related NTDs associated with maternal opioid exposure could provide insights into biomarkers and/or pathways underlying opioid-related birth defects.

Introduction

Findings from independent reports[1,2] found an increased risk for NTDs in babies born to mothers who had used opioids. This is an important healthcare concern due to increasing[3] rates of opioid use during pregnancy, which is reported as 7 to 14% of women[4,5]. FDA released a Drug Safety Communication in 2015 urging careful consideration when taking any pain medicines during pregnancy, but new recommendations for opioid use during pregnancy were not provided due to limitations in human and animal study findings. The mechanism behind the link between fetal opioid exposure and NTDs is still not fully understood.

An animal study was conducted to investigate hypoxia as a possible mechanism of opioid induced NTDs following maternal mouse exposure to methadone and morphine. Multiple markers for hypoxia were measured and analyzed. Our study used high resolution MALDI-IMS combined with pathology analysis of sequential fetal mouse tissue sections to assess changes in lipid spatial distribution, particularly in the brain and central nervous system (CNS). Data was then used to investigate potential mechanisms of opioid induced NTDs following maternal exposure to methadone or morphine.

Materials and Methods

1. Pregnant CF-1 mice were treated with a single dose of vehicle or a low or high dose of methadone (10 and 30 mg/kg BW), morphine (100 and 400 mg/kg BW), or positive control VPA (300 and 500 mg/kg BW) on gestational day (GD) 8 (**Figure 1**).
2. On GD8, blood-pressure, ultrasound of uterine artery blood flow, and blood gas parameters preliminarily suggested evidence of hypoxic conditions in the dams.
3. Dams were sacrificed on GD18 and fetuses were harvested and frozen for MALDI IMS to screen for changes in lipid distribution.

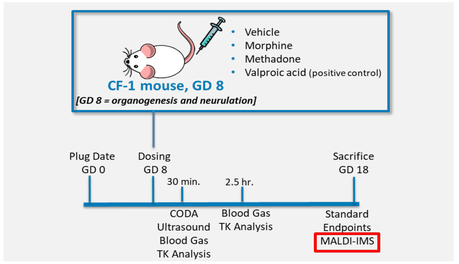

Figure 1. Overview of the study design for assessment of fetal exposure to opioids during neural tube development

4. Frozen fetuses were prepared for staining and MALDI IMS (**Figure 2**):
   A. Fetuses were halved along the midsagittal plane, and 12 µm sections were removed and mounted on slides for staining and MALDI IMS.
   B. Sections for staining were fixed in 1:1 methanol/acetonitrile and stained with H&E.
   C. Sections for MALDI IMS were spray coated with 2,5-dihydroxybenzoic acid (DHB) matrix in 70% methanol and 0.1% TFA.
   D. MALDI slides were imaged using a Bruker scimaX 7T FTMS. A low resolution scan (raster width, 100 µm) targeted broad range of masses (*m/z* 100-1500) to screen for overall lipid distribution changes in the whole mouse fetuses.
   E. Data was loaded in FlexImaging for image visualization and analysis. Lipid identities were assigned based on parent mass (*m/z*) and confirmed using CID for analyte fragmentation.

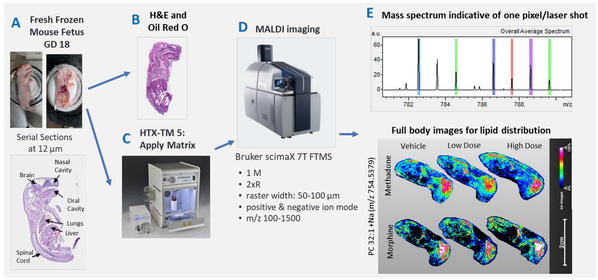

Figure 2. Method overview of mouse fetus preparation for staining and MALDI IMS.

Results and Discussion

Overall screening of common brain lipids, including twelve linked to hypoxia, revealed four types of lipids that visibly changed between treatment groups (**Table 1**). All changes were localized to the brain thalamus and hypothalamus regions. These lipid distributions were differential between doses, and trends were consistent between methadone (**Figure 3**) and morphine (**Figure 4**) exposed fetuses. Lyso PC 16:0, PC 32:1, and PC 34:2 levels increased with opioid exposure while PC 34:1 levels decreased. These trends were also observed in VPA exposed fetuses (data not shown), which served as the positive control for the study.

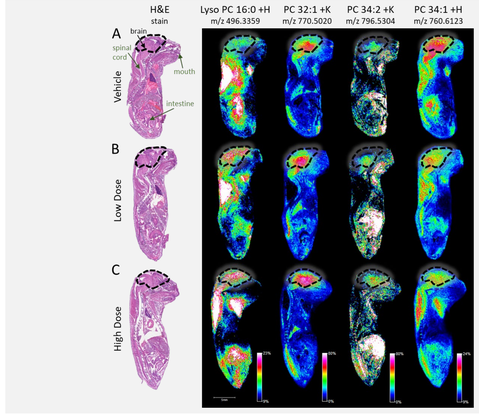

Figure 3. Imaging of whole sagittal sections of methadone exposed mouse fetuses. Sagittal sections (12 µm) were taken from mouse fetuses exposed to vehicle control (A), low dose methadone at 10 mg/kg BW (B), and high dose methadone at 30 mg/kg BW (C).

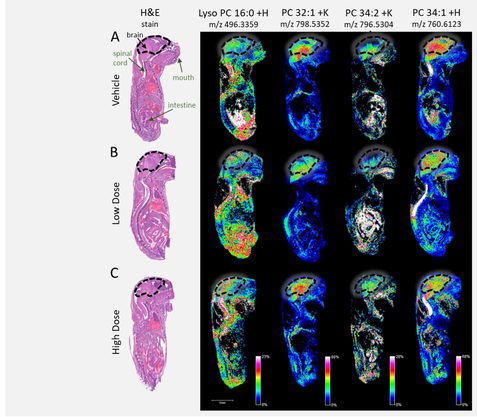

Figure 4. Imaging of whole sagittal sections of morphine exposed mouse fetuses. Sagittal sections (12 µm) were taken from mouse fetuses exposed to vehicle control (A), low dose morphine at 100 mg/kg BW (B), and high dose morphine at 400 mg/kg BW (C).

Table 1. Lipid distribution changes observed for methadone and morphine exposed mouse fetuses

lipid	adducts observed[a]	parent m/z	observed change[b]
Lyso PC 16:0	+ H	496.3391	increased
	+ Na	518.3150	increased
	+ K	534.2892	increased
PC 32:1	+ Na	754.5237	increased
	+ K	770.5020	increased
PC 34:2	+ K	796.5130	increased
PC 34:1	+ Na	784.5802	decreased
	+ K	798.5489	decreased

[a] Adduct identities were confirmed using CID to yield fragments at m/z 162 for K adducts and m/z 184 for Na adducts
[b] Changes were qualitatively observed in the brain for opioid exposed fetuses relative to vehicle exposed fetuses

Conclusion

- Increases in PC 34:2 and PC 34:1 are both linked to hypoxic conditions[6]. However, we observed an increase in the former with opioid exposure, while a decrease was observed for the latter. Thus these apparently conflicting preliminary results may not support hypoxia as the underlying mechanism of NTDs resulting from opioid exposure.
- Lyso PC 16:0 level increases were consistent for multiple adducts (+H, +Na, +K) and were observed with both opioids. This lipid has a known link to neurodegenerative conditions[7], suggesting that this pathway could be involved in the molecular mechanism for opioid-induced NTDs.
- These low resolution scans demonstrated the useful application of MALDI IMS for imaging lipid distributions in whole mouse fetuses, which revealed useful targets for future high resolution scans.
- Future high resolution scans will image mouse fetal coronal sections targeting the thalamus and hypothalamus for a more thorough and detailed assessment of lipid distribution changes in response to opioid exposure. Images will be assessed using Bruker's SCiLS software for quantitative and statistical analysis of observed lipid changes.

References:

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