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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) based on previous reports. At the time, FDA did not make new recommendations for opioid use during pregnancy due to incomplete maternal toxicity data and limitations in human and animal studies. Since then, FDA scientists have conducted multiple comprehensive studies designed to determine whether opioid-induced maternal toxicities, including hypoxia, are associated with fetal NTDs. In this study, lipid changes in mouse fetuses following exposure to morphine (400 mg/kg body weight (BW)), the positive control valproic acid (VPA) (500 mg/kg BW), or the vehicle negative control 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 sections of an organ or whole-body. Following maternal exposure to the test articles on gestational day (GD) 8, whole-body mouse fetal sagittal sections with and without NTDs were analyzed using MALDI-IMS on GD 18. Differential lipid distributions related to dose and exposure were identified for several phosphatidylcholine (PC) classes in the fetal brains, including PCs 34:1, 34:0, and 36:2, all of which have been previously associated with hypoxia. Follow-up high resolution imaging of horizontal sections revealed regional increases and decreases in PC levels in the cerebral cortex, thalamus, hypothalamus, and hippocampus. Additionally, an increase in the distribution of lyso PC 16:0 was observed across the brain with drug exposure. Lipid identities were confirmed with collision-induced dissociation (CID) for analyte fragmentation. MALDI images were also aligned to hematoxylin and eosin (H & E) staining of serial sections to map these distributions to histopathology. To the best of our knowledge, these findings represent the first MALDI-IMS study of whole-body fetuses with opioid exposure during gestation. The observed changes in lipid distribution within fetal neural tissues suggest that hypoxic conditions are linked to maternal drug exposure; however, there is no clear correlation with NTD occurrence. Thus, a possible link between hypoxia and drug-induced NTDs requires further investigation.

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 rates[3] of opioid use during pregnancy, which are reported as 7 to 14% in women[4,5]. FDA released a Drug Safety Communication in 2015 urging careful consideration when taking any pain medicines during pregnancy[6], 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 morphine and the positive control VPA, which has established links to exencephaly development[7]. 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. Data was then used to investigate potential mechanisms of opioid-induced NTDs following maternal exposure to morphine.

Materials and Methods

1. Pregnant CF-1 mice were treated with a single dose of vehicle (saline), morphine (400 mg/kg BW), or VPA (500 mg/kg BW) on GD 8 (**Figure 1**).
2. On GD 8, 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 GD 18 and fetuses were harvested and frozen for MALDI-IMS to screen for changes in lipid distribution.

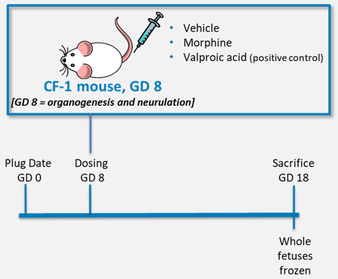

Figure 1. Overview of the study design for assessment of fetal exposure to opioids during neural tube development.

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 whole-body scan (raster width, 100 µm) targeted a 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.

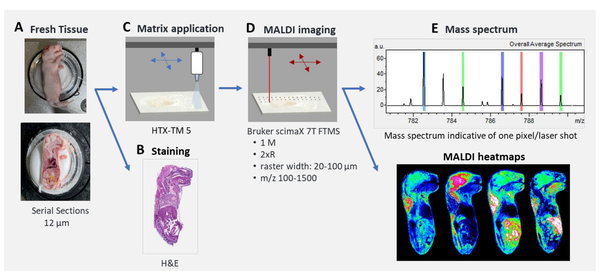

Figure 2. Method overview of mouse fetus preparation for staining and MALDI-IMS.

Results

Overall screening of common brain lipids, including twelve linked to hypoxia, revealed four types of lipids that visibly changed between treatment groups (**Table 1**). The differential lipid distributions between fetal groups were observed visually in the MALDI heatmaps (**Figure 3**) as well as graphically from plotted pixel point intensities using SCiLS software (**Figure 4**). PC 34:0, 36:2, and 34:1 levels increased with morphine exposure for both fetal groups, while only lyso PC 16:0 levels increased more so in the exencephaly group than in the morphine-exposed group without exencephaly. Similar trends were observed for VPA-exposed fetuses (data not shown).

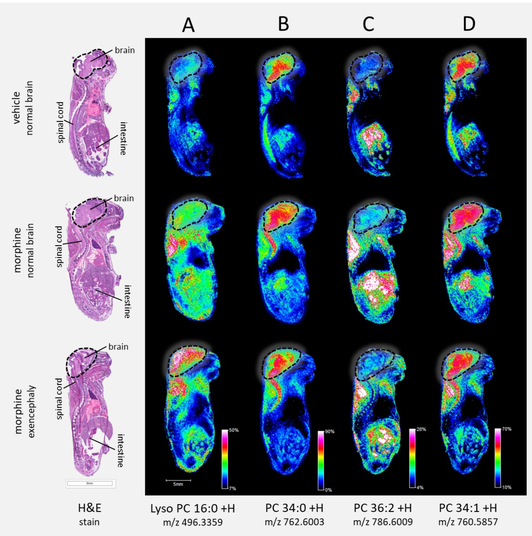

Figure 3. Imaging of whole sagittal sections of morphine-exposed mouse fetuses. Heatmap images (FlexImaging) of sagittal sections (12 µm) taken from mouse fetuses exposed to vehicle or morphine with normal brain development or exencephaly show differential lipid distributions within the brains for (A) lyso PC 16:0 and PCs (B) 34:0,(C) 36:2, and (D) 34:1.

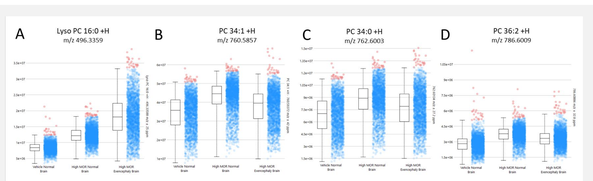

Figure 4. Qualitative analysis of lipid distribution changes for morphine exposed-mouse fetuses. Lipid distribution assessments of fetal brains using SCiLS (**Figure 3**) show differential distributions between treatment groups for (A) lyso PC 16:0 and PCs (B) 34:0, (C) 36:2, and (D) 34:1. Each dot represents the signal intensity at a single pixel in the MALDI image, with red dots representing outliers (1.5 x interquartile range).

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

lipid	adducts observed[a]	observed parent (m/z)	LIPID MAPS reported (m/z)	mass accuracy (ppm)	CID fragment	observed trend[b]
Lyso PC 16:0	+ H	496.3394	496.3398	-0.805899507	184.07	Increased with drug exposure and exencephaly
	+ Na	518.3219	518.3217	0.38586075	146.98	
	+ K	534.2959	534.2956	0.561486937	162.96	
PC 34:0	+ H	762.6003	762.6007	-0.524520893	184.07	Increased with drug exposure
	+ Na	784.5829	784.5827	0.25491258	146.98	
	+ K	800.5568	800.5566	0.249826183	162.96	
PC 36:2	+ H	786.6009	786.6007	0.254258609	184.07	Increased with drug exposure
	+ K	824.5565	824.5566	-0.121277302	162.95	
PC 34:1	+ H	760.5857	760.5851	0.788866361	184.07	Increased with drug exposure
	+ K	798.5403	798.5410	-0.876598697	162.95	

[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 between fetal groups

Discussion

- Changes in PCs 34:0, 36:2, and 34:1 are all linked to hypoxic conditions[8]. The observed increases with drug exposure support fetal brain hypoxia as a possible outcome of maternal morphine exposure; however, no apparent differences were observed between normal brains and brains with exencephaly. Thus, a possible link between hypoxia and morphine-induced exencephaly is still unclear.
- Lyso PC 16:0 level increases were consistent for multiple adducts (+H, +Na, +K) and were observed with both drugs. This lipid has a known link to neurodegenerative conditions[9], suggesting that this pathway could be involved in the molecular mechanism for opioid-induced NTDs.
- These sagittal 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 higher-resolution scans will image mouse fetal coronal sections 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 analysis of observed lipid changes and aligned with serial sections for pathology analysis using Cresyl violet and Fluoro-Jade C staining.

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