Cross Platform Lipid Imaging of Silver Nanoparticle-Exposed Rat Brain by LAESI-MS and MALDI-MS

Callee Walsh[1]; Pamela Cantrell[1]; Krishnan Sriram[2]; Jenny R. Roberts[2]; Erin H. Seeley[1]; Katherine Moss[1]; Peggi Angel[1]

[1]Protea Biosciences, Morgantown, WV; [2]CDC-NIOSH, Morgantown, WV

Overview

LAESI and MALDI imaging performed on brain samples to compare lipids detected.

Lipid species detected highlight complementarity of techniques.

Effects of silver nanoparticle exposure were evaluated using imaging modalities.

Silver nanoparticles (AgNP) are widely used in industrial, household, diagnostic and antimicrobial products. Emerging studies suggest that AgNP can biodistribute to the brain following inhalation and potentially be neurotoxic. However, the molecular effects of AgNP exposure have not been fully explored.

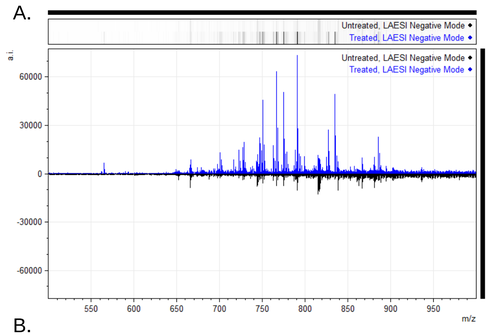
Here, we report the utility of differing MSI platforms in identifying rat brain lipidome changes following AgNP inhalation. The use of multiple mass spectrometry imaging (MSI) platforms has the advantage of both increasing and cross-validating the information gathered from individual MSI experiments. Lipid data are generated by classical matrix-assisted laser desorption/ionization (MALDI) and laser ablation electrospray ionization (LAESI) MSI platforms and illustrate complementary signatures between platforms. These data increase our understanding of possible health effects due to AgNP in common products.

Methods

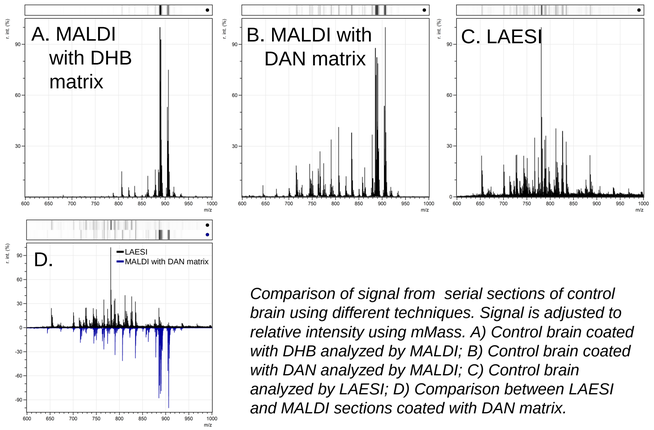
Male Sprague-Dawley rats were exposed by inhalation to 1 mg/m^3 AgNP (20 nm diameter, 0.3% wt/wt PVP coating) or filtered air (control) for 14 week days (4 h/d) and sacrificed 28 d post exposure. Brains were cryopreserved and sectioned.

For MALDI, 12 µm sections were washed, spray-coated with DHB or DAN using SunCollect (SunChrom), imaged by MALDI TOF (ultrafleXtreme, Bruker), and processed by flexImaging (Bruker).

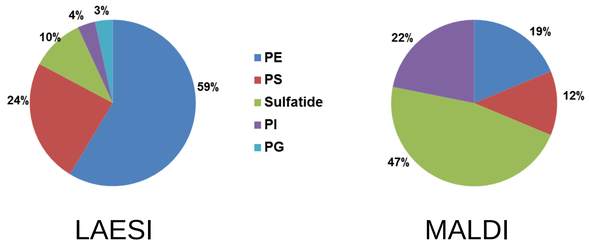
For LAESI, 30 µm sections were washed, analyzed using LAESI DP-1000 coupled to an ion mobility mass spectrometer (Synapt G2 S, Waters) and the image data was normalized using ProteaPlot (Protea Biosciences). All imaging was performed in negative ion mode. Lipids were identified by accurate mass, MS/MS, and/or collision cross-sections compared to available databases.



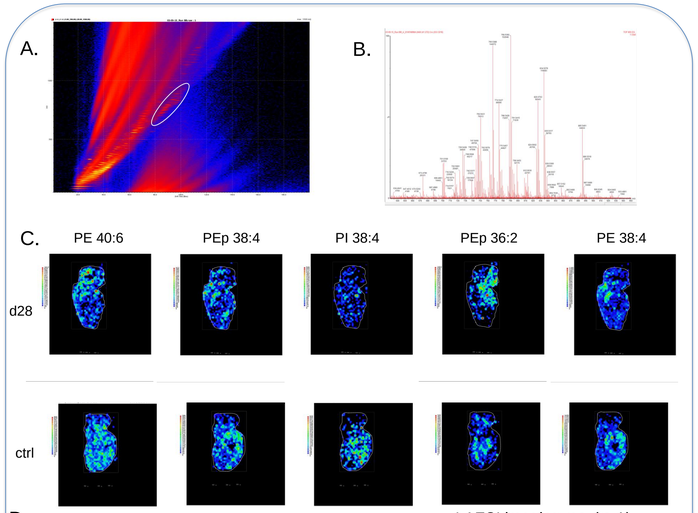
Comparison of signal from washed (50 mM cold ammonium formate, 4X) and unwashed tissue using LAESI. A) Average spectra from washed (upper) and unwashed (lower) brain tissue. B) Selected images of lipid species indicating the benefit of washing (left washed, right unwashed).



Comparison of signal from serial sections of control brain using different techniques. Signal is adjusted to relative intensity using mMass. A) Control brain coated with DHB analyzed by MALDI; B) Control brain coated with DAN analyzed by MALDI; C) Control brain analyzed by LAESI; D) Comparison between LAESI and MALDI sections coated with DAN matrix.

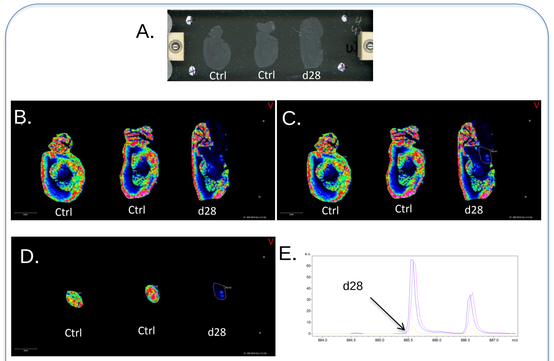


Comparison of lipid classes detected in LAESI and MALDI experiments (%).

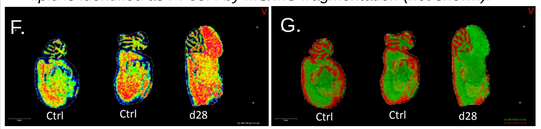


Lipid ID	Theoretical m/z	Observed m/z	Δppm	Observed CCS	CCS % Diff
PE 40:6	790.5392	790.5392	<0.1	289.10	-0.5
PE 38:4	766.5392	766.5388	0.5	284.36	-0.2
PEp 38:4	750.5443	750.5431	1.6	284.49	-0.8
PI 38:4	885.5498	885.5491	0.8	309.35	-0.6
PEp 36:2	726.5443	726.5438	0.7	276.97	-0.9

LAESI imaging results A) Driftogram from LAESI-MS analysis with lipids circled. B) Average spectrum from ctrl C) selected LAESI images from ctrl and d28 D) Table of selected lipids with identification confirmed by CCS value.



MALDI images from control and d28 brain exposure samples. A) Photo of brain sections B) m/z 885.6 C) m/z 885.6 with ROI selected D) m/z within ROIs E) spectral comparison for m/z 885.6 indicates a potential reduction in m/z 885.6 due to exposure. This lipid is identified as PI 38:4 by MS/MS fragmentation (not shown).



MALDI images from control and d28 brain exposure samples. F) m/z 890.7 G) image overlay of m/z 890.7 (green) and 885.6 (red).

Conclusions

- LAESI spectra are enriched in PE and PS species.
- MALDI spectra are enriched in sulfatides and PI species.
- Preliminary imaging analyses may indicate a decrease in PI 38:4 and an increase in PE40:6 and PEp 36:2 at d28 post-exposure to silver nanoparticles.
- Use of both imaging modalities provides cross-validation and complementarity to better understand biological differences.