Accumulation of Bioactive lipids in LPS-induced Neuroinflammation models: MSI Biomarkers

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In-vivo experiments

Rat models were treated for 24 and 48 hours with Lipopolysaccharides (LPS) from Escherichia coli to induce neuroinflammation and trigger glial inflammation. LPS promotes the activation of "classically activated" M1-type microglia[1].

Inflammatory process induced with LPS

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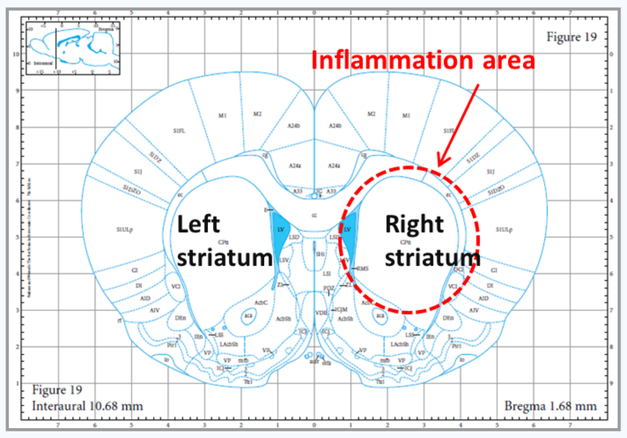
Microglial activation

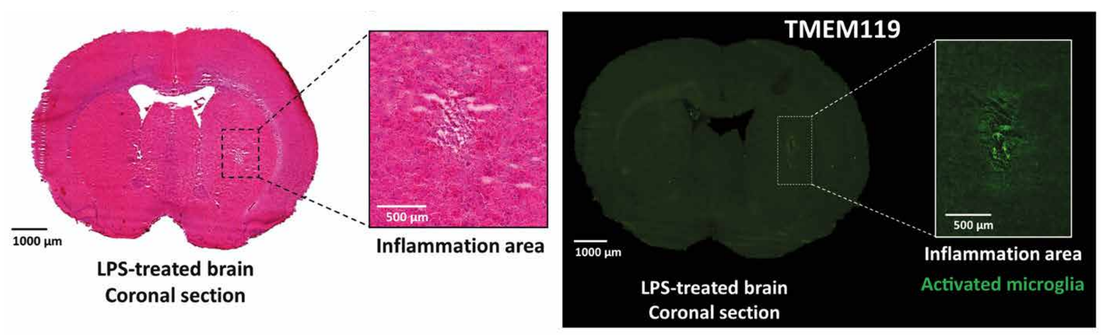


Stereotactic injection of LPS in the right striatum via a craniotomy (bregma +0.7 mm, lateral −3.0 mm, depth 5.5 mm the brain)[2].

Histological staining and Immunofluorescence

A H&E staining

B Immunofluorescence



A) H&E staining applied to coronal sections for the identification of the inflammation area.
B) Immunofluorescence staining showing the areas where TMEM119 binds to activated microglia. The presence of microglia can be seen as fluorescent green.

Objective

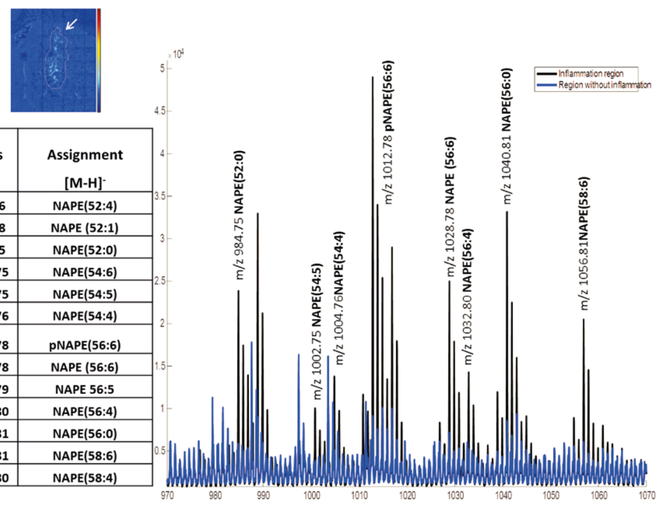
Mass Spectrometry Imaging (MSI) was applied to the analysis of neuroinflammation models. We aim to identify active lipid species that accumulate during the inflammation process and the possible ion signatures associated with the presence of activated microglia cells. Multimodal imaging techniques were applied such as MALDI, DESI, TOF-SIMS and microscopy.

Multi-modal imaging

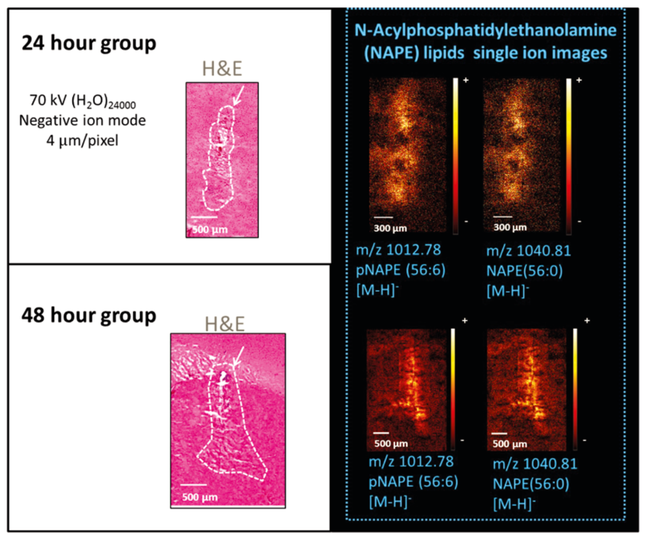
Mass Spectrometry Imaging (MSI)

TOF SIMS

Inflammation region vs surrounding tissue spectra overlay (24 hrs)

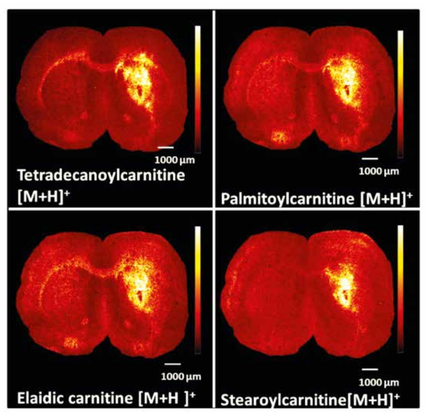


Mass (Da)	Assignment [M-H]⁻
976.76	NAPE(52:4)
982.78	NAPE (52:1)
984.75	NAPE(52:0)
1000.75	NAPE(54:6)
1002.75	NAPE(54:5)
1004.76	NAPE(54:4)
1012.78	pNAPE(56:6)
1028.78	NAPE (56:6)
1030.79	NAPE 56:5
1032.80	NAPE(56:4)
1040.81	NAPE(56:0)
1056.81	NAPE(58:6)
1060.80	NAPE(58:4)



Spectra from the inflammation region revealed chemical differences between this area and the rest of the tissue (top image). NAPE lipids are specifically accumulated within the area of inflammation where activated microglia was observed

MALDI



Images showing the distribution of Fatty Acyl Carnitines detected with MALDI. Fatty acyl carinitines accumulate around the inflammation region. MALDI images are compared to H&E indicating the inflammation area.

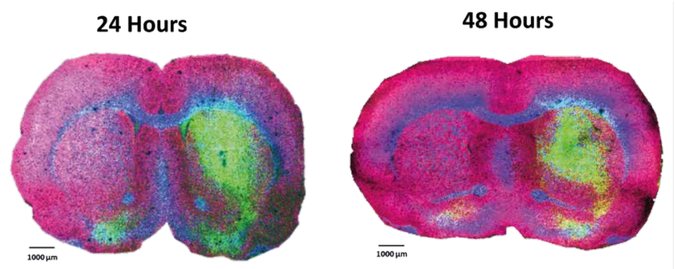
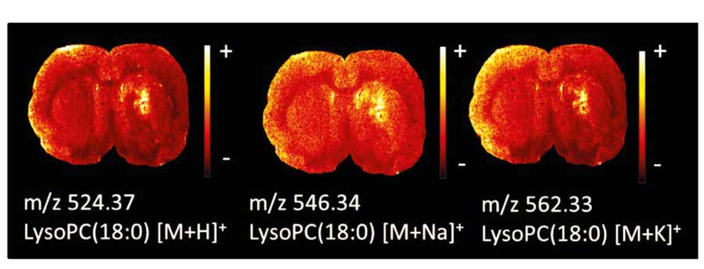
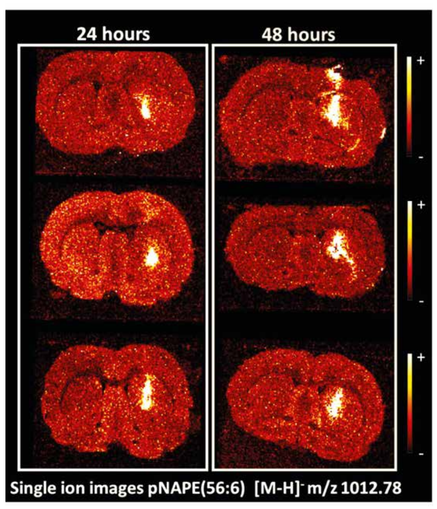


Image overlay showing accumulation of Palmitoylcarnitine around inflammation- Palmitoylcarnitine; PC(34:1) Grey matter, HexCer(t42:1) White matter.



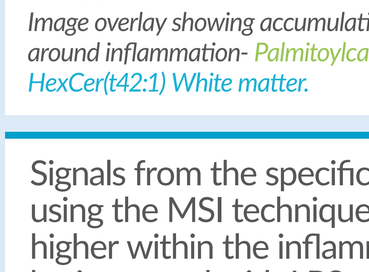
MALDI Imaging was applied to the identification of Fatty Acyl Carnitines, Lysophospholipids and Ceramides upregulated around the region of inflammation. Above– single ion images showing the distribution of LysoPC(18:0) around the inflammation area.

DESI (-)



Detection of N-acyl phosphatidylethanolamines with DESI in negative ion mode confirmed the distribution of these lipid species within the inflammation region where activated microglia was observed.

DESI (+)



Upregulation of fatty acyl carnitines around the area of inflammation was observed with DESI in positive.

Image overlay showing accumulation of Palmitoylcarnitine around inflammation- Palmitoylcarnitine; PC(34:1) Grey matter, HexCer(t42:1) White matter.

Signals from the specific molecules identified using the MSI techniques were found to be higher within the inflammation regions of the brain treated with LPS and are therefore thought to have the potential to act as biological active lipids during the inflammation processes.

With results from all three imaging modalities showing promising correlation of molecular ions to neuroinflammation, future experiments will investigate the potential for use of these markers in translational studies for drug discovery. This may include repeating the analysis in human cell models and human tissue to show translation from in-vivo rodent studies to human. These markers may then be monitored in response to therapeutic interventions.

References

[1]Hong J, Yoon D, Nam Y, et al. Lipopolysaccharide administration for a mouse model of cerebellar ataxia with neuroinflammation. Sci Rep. 2020;10(1):1-10. doi:10.1038/s41598-020-70390-7

[2]Paxinos G, Watson C. Paxino's and Watson's The Rat Brain in Stereotaxic Coordinates. 7th ed.; 2013.

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