Investigating Cholesterol in Brain by AP-MALDI Mass Spectrometry Imaging

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Introduction

Cholesterol is an essential molecule for health. In the central nervous system it is vital for normal neuronal and glial function. Cholesterol and sterol dysregulation have been linked to several neurodegenerative diseases including Alzheimer's (AD)[1], Huntington's (HD)[2] and multiple sclerosis[3].
Most of the conventional methods for sterol analysis from tissue samples are by tissue homogenisation followed by lipid extraction and analysis by mass spectrometry (MS). However, homogenisation looses all structural information from the tissue. In addition, cholesterol being a difficult molecule to ionise means the MS spectra may not accurately represent the true amount of endogenous cholesterol.
Mass spectrometry imaging (MSI) allows the analysis of lipids across intact tissue sections for precise analysis in pathological and anatomical regions of interest.
Matrix-assisted laser desorption/ionization (MALDI)-MSI is fast becoming one of the most popular qualitative methods for lipid analysis, using a laser to desorb and ionize material from the tissue surface with the help of a matrix layer. Despite cholesterol being a critical molecule in the CNS and reports of its dysregulation being linking to neurodegeneration, MSI of cholesterol has not fully been explored primarily due to the difficulty of ionizing cholesterol.

Aims

The main aim of this project is to optimise an atmospheric pressure (AP)-MALDI-MSI method to allow for the quantification and visualization of cholesterol across different neurodegenerative disease and control brain tissue sections exploiting on-tissue enzyme-assisted derivatisation to enhance sensitivity.

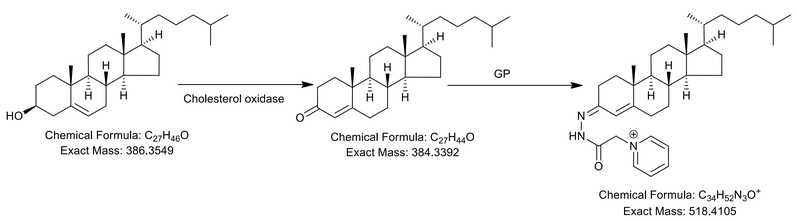
Methods and Materials

Brain tissue

Q150$^+$ transgenic mice and corresponding controls as well as human post-mortem multiple sclerosis and control brain tissue was obtained with ethical permission. All tissue sections were snap-frozen, cut at 10 µm thickness and mounted onto indium tin oxide (ITO) coated glass slides.

On tissue derivatisation method

To overcome the poor ionisation properties of cholesterol, we exploited on-tissue derivatisation to charge-tag cholesterol before analysis with MALDI[4]. We also sprayed on a deuterated internal standard ([$^2H_7$]cholesterol) which allowed us to quantify the amount of endogenous cholesterol present in the brain.



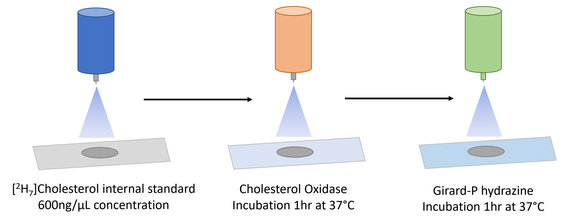


Figure 1. Slide preparation for MALDI analysis, using deuterated internal standard and an on-tissue enzyme derivatisation method[5].

MALDI analysis

The matrix used for all experiments was α-cyano-4-hydroxycinnamic acid (CHCA). An AP-MALDI source from MassTech was interfaced to an Orbitrap IDX from Thermo Scientific. For each 50 µm laser spot, one mass spectrum was created showing the ions desorbed from that specific point. This was done across the whole brain, allowing an image to be created (shown in Fig 2; normalised image of cholesterol against internal standard in sagittal mouse brain).

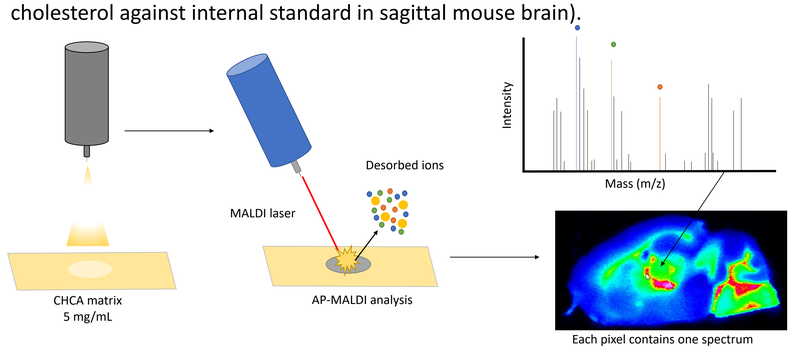
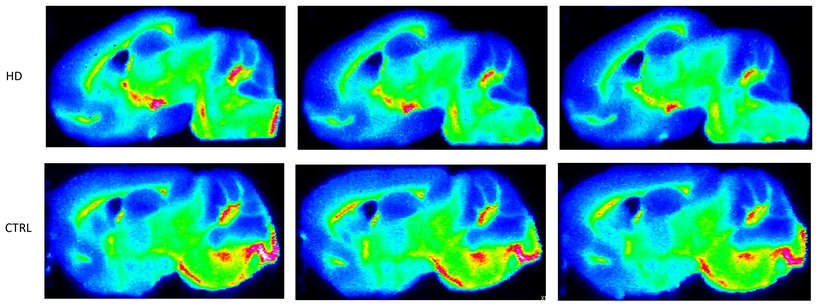


Figure 2. Analysis of tissue section using AP-MALDI-MSI. After matrix was sprayed across the derivatised tissue section, the ITO-coated slide was placed into the AP-MALDI source and irradiated to desorb ions from the tissue. A mass spectrum for each pixel was generated. All the spectra combined allow for an image to be created using Multimaging software of a chosen ion according to its exact mass.

Results

Huntington's disease Q150$^+$ mouse models

HD is a recessive neurodegenerative disease characterised by a progressive loss of neurones, starting in the striatum of the brain. Some earlier studies using micro-puncturing suggest cholesterol to be increased in the striatum of mouse models of HD and in the clinical disease[2]. We sought to determine the concentration of cholesterol in wild type and Q150$^+$ transgenic mice using our MSI method.



Average absolute amount of cholesterol in the striatal region (ng/mm^2)						
HD	sec1	sec2	sec3	sec4	sec5	sec6
	396.0	312.0	308.4	306.0	307.2	310.8
Control	sec1	sec2	sec3	sec4	sec5	sec6
	447.6	373.2	405.6	376.8	393.6	385.2

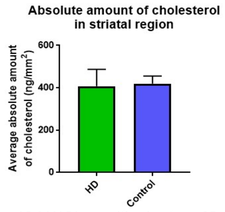


Figure 3. No change in cholesterol witnessed between HD and control striatum in the brain . AP-MALDI quantification from 35 brain sections (18 HD; 17 control; examples of 6 images shown above) show the absolute amount of cholesterol in the striatal region of mouse brain is comparable between control and HD mice (n=1 for each mouse model). The MALDI images were imaged at 50 µm resolution.

We have successfully imaged cholesterol in WT and transgenic mouse brain, with this data comparable to other published work[4]. Preliminary analysis from one disease and one control mouse brain show that the cholesterol within the striatal region of both mouse brains was very similar, with no large difference in the data. More data and multiple mouse brains need to be analysed before we can say there was no significant difference.

Multiple sclerosis human brain tissue

Multiple sclerosis is a neurodegenerative, autoimmune disease, centring around inflammatory demyelination.
Cholesterol is highly enriched in the brain and the main component of myelin, so we wanted to look at how demyelination and additional inflammation in the human multiple sclerosis brain affected cholesterol in these regions of interest (ROI).

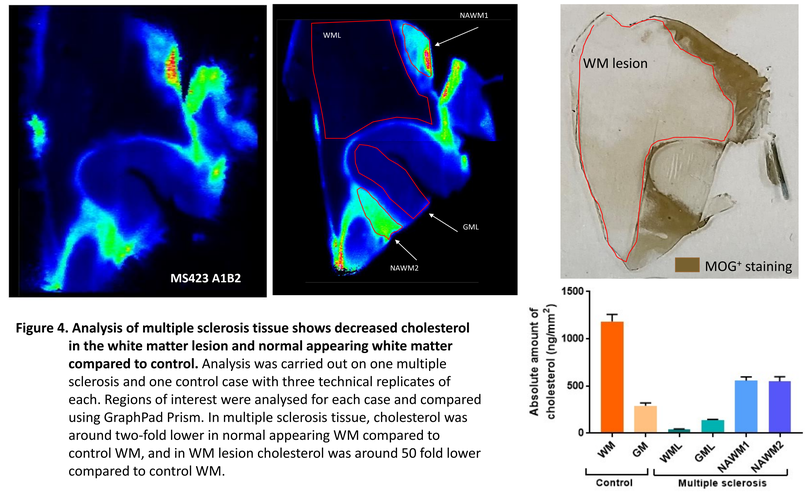


Figure 4. Analysis of multiple sclerosis tissue shows decreased cholesterol in the white matter lesion and normal appearing white matter compared to control. Analysis was carried out on one multiple sclerosis and one control case with three technical replicates of each. Regions of interest were analysed for each case and compared using GraphPad Prism. In multiple sclerosis tissue, cholesterol was around two-fold lower in normal appearing WM compared to control WM, and in WM lesion cholesterol was around 50 fold lower compared to control WM.

Preliminary data from one disease and one control human brain shows that cholesterol in specific ROI's within multiple sclerosis tissue differs from control tissue. In multiple sclerosis, the WM lesion and normal-appearing WM have much lower cholesterol compared to control WM, showing a 50-fold and 2-fold decrease respectively. This fold change suggests a trend, however, more analysis needs to be done using other multiple sclerosis and control cases to confirm reproducibility between cases.

Conclusion

We have optimised a reproducible method for visualisation and quantification of cholesterol across tissue sections using AP-MALDI-MSI. This method has allowed us to explore both HD mouse models and human multiple sclerosis tissue.

Future work and other applications

Although the focus for this project has been mapping of cholesterol specifically, other lipids, such as ceramides and sphingolipids, can be mapped in the same way with AP-MALDI-MSI and the hope is to identify and visualise other lipid classes which associate spatially with disease pathology
We have preliminary data for analysing cholesterol across multiple sclerosis human tissue sections, however, multiple sclerosis is a complex disease with many pathological features and varying levels of those pathologies. Therefore the next steps of this project are to analyse other cases with differing pathology to get more insight to whether these pathological changes affect cholesterol within the multiple sclerosis brain.

References

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