LIPID METABOLISM IN TRAUMATIC BRAIN INJURY – INCREASED IDENTIFICATION CONFIDENCE WHILST EXTENDING COVERAGE WITH A MULTI-REFLECTING TIME OF FLIGHT MASS SPECTROMETER

Eszter Ujvari[1], Rita Campos-Pires[1], Lee A. Gethings[2], Elizabeth J. Want[1], Robert Dickinson[1]

[1]Department of Surgery & Cancer, Imperial College, London, UK; [2]Waters Corp., Wilmslow, Cheshire, United Kingdom

INTRODUCTION

A rat model of TBI was utilized, consisting of a sham cohort (no injury) and TBI cohort (treated with control gas or xenon[1]). Treatment began 30 min after injury over a 3-hr duration to model the scenario of treatment by first responders. Samples of brain tissue were taken at 3 and 24 hrs in addition to a sham at the same timepoints with control gas only. Lipids were extracted from plasma using a folsch procedure and diluted appropriately for LC-MS analysis. Lipids were separated over a 6-min gradient using a U(H)PLC configured for reversed-phase (RP) chromatography. The LC was interfaced with a multi-reflecting time-of-flight mass spectrometer (MS) and data collected with a data independent (DIA) and data dependent (DDA) mode of acquisition prior to being processed and database searched. Features with a mass accuracy of sub 1 ppm (precursor and fragment ions) and CV of <20% (based on study QC reproducibility) were used for further multivariate statistical analysis (MVA) to establish differences between groups. The data inherently showed that the lipidome is significantly affected after TBI, with clear differences between xenon and control gas.

OVERVIEW

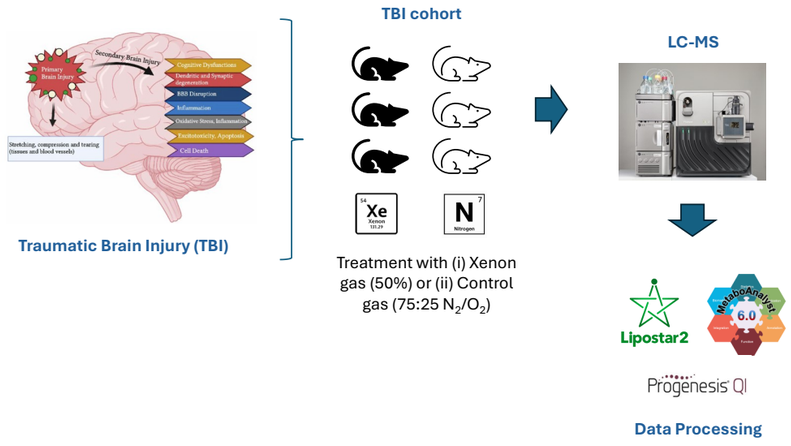


Figure 1. Experimental workflow - Mouse models (also including controls, which have TBI but do not undergo any treatment) with TBI are treated with either xenon gas or control gas. Blood samples were taken from all subjects with lipids and polar metabolites extracted for LC-MS analysis. The subsequent data were then processed using a variety of software packages with additional statistical analysis being performed with Metaboanalyst.[2,3]

Blood samples taken at various time points (i.e., 3 & 24 hrs) from all three groups were prepared for LC-MS analysis, using a folsch based method to obtain lipids and polar metabolites. Components were separated using an ACQUITY™ Premier UPLC™, configured initially with a 2.1 mm (i.d.) column based on a 10 min gradient. This was later adapted to a 5 min gradient to utilize the acquisition speed of the MS and increase sample throughput. A Xevo™ MRT MS was used as for data collection, acquiring data as DIA or DDA with scan rates of up to 50 Hz (MS) and 100 Hz (MS/MS). Representative MS spectra for an example lipid identified during LC method optimization is provided in figure 2. In addition to lipidomic analyses, polar metabolite data were also collected using a short chromatographic method (HILIC based). Exemplar positive ion data is provided in figure 3, indicating differences across the various groups analyzed.

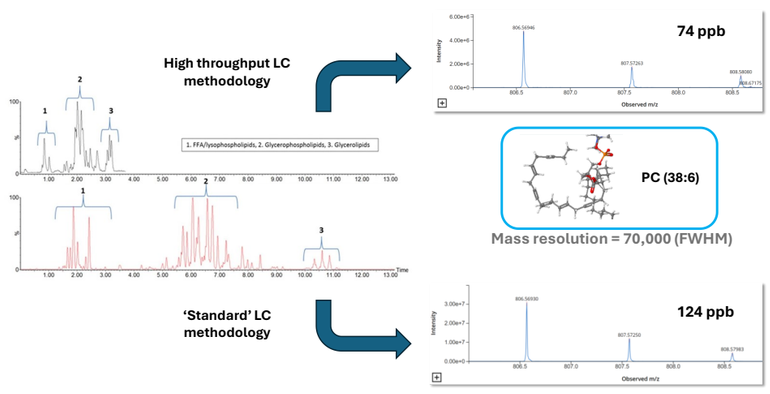


Figure 2. Method optimization to increase sample throughput. Original 12 min lipid method was reduced to <4 min using the same 2.1 mm RP column. The example lipid (PC(38:6)) shows highlights that instrument performance is maintained even with short chromatographic gradients. Mass resolution is maintained (~70,000 FWHM) as well as mass accuracy (i.e., 74 ppb for the faster chromatography).

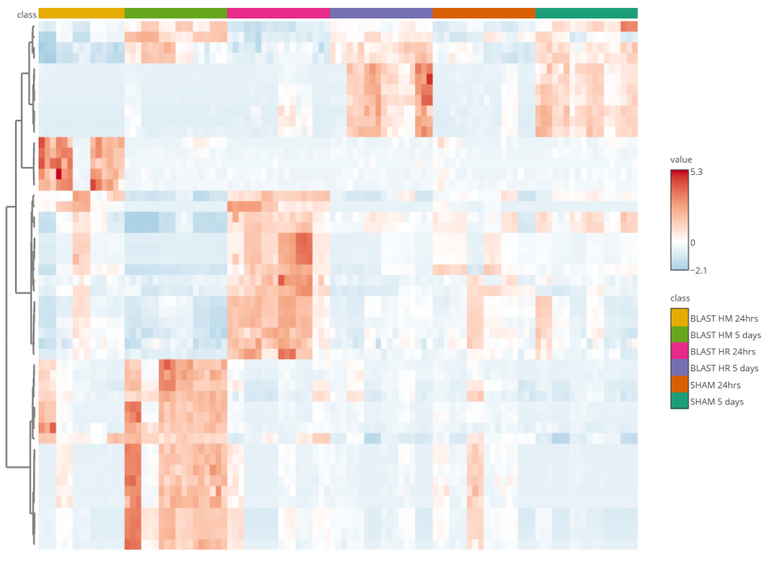


Figure 3. Heatmap for the top 100 polar metabolite features (ESI+) based on t-test/ANOVA. The plot represents the various groups across the two time points (3 hr and 24 hr).

RESULTS

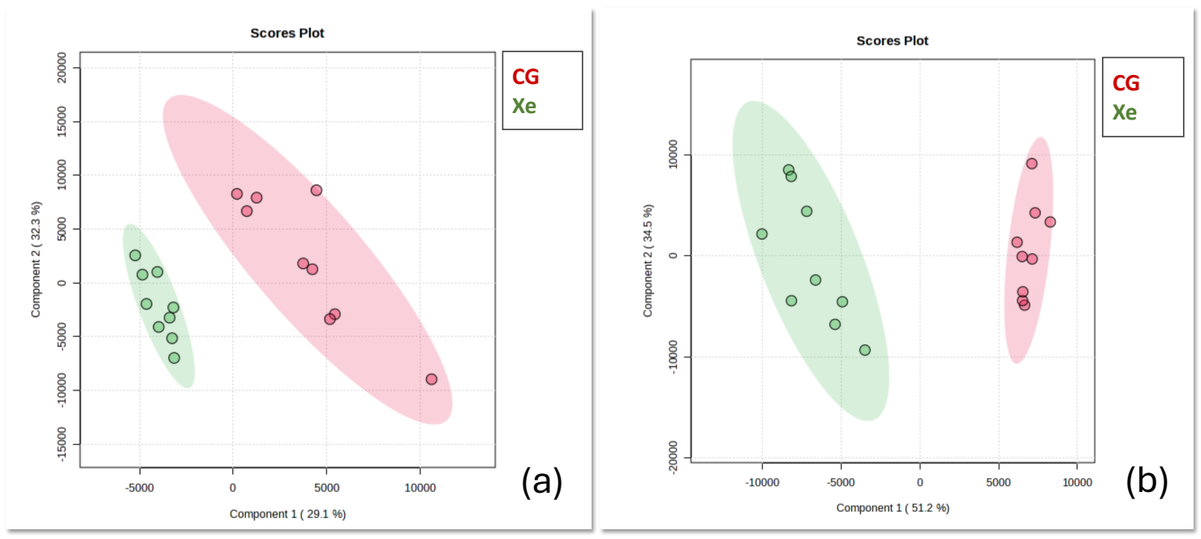
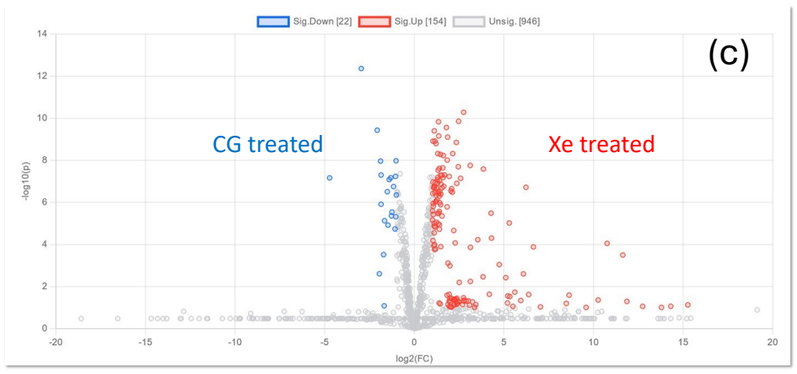


Figure 4. Unsupervised principal component analysis (PCA) highlighting clear separation of lipid features between the control gas treated group and xenon treatment over a time course, which include 3 hrs (a) and 24 hrs (b). The resulting volcano plot (c) indicates the lipid features which significantly contribute to the differentiation between control gas and xenon treatment at 24 hrs. Highlighted features signify a log fold change (≥ 2) and ANOVA (p) value ≤ 0.05.



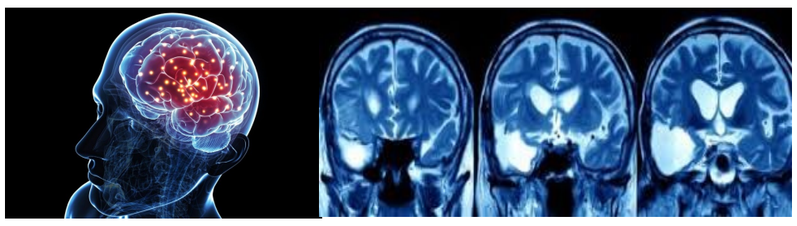
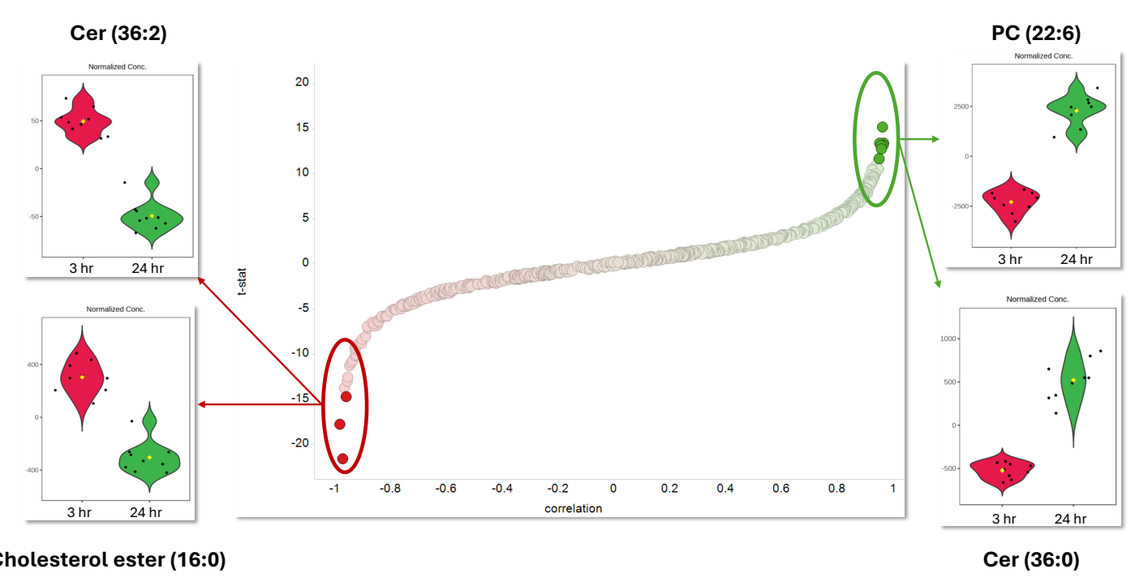


Figure 5. Unsupervised principal component analysis (PCA) highlighting clear separation of lipid features between the control gas treated group and xenon treatment over a time course, which include 3 hrs (a) and 24 hrs (b). The resulting volcano plot (c) indicates the lipid features which significantly contribute to the differentiation between control gas and xenon treatment at 24 hrs. Highlighted features signify a log fold change (≥ 2) and ANOVA (p) value ≤ 0.05.

Various multi-variate statistical approaches were taken during data analysis, including principal component analysis (PCA) and identification of features for subsequent compound identification from volcano plots (figure 4). Changes in lipid distribution are provided via correlation analysis (figure 5) with a variety of lipid classes being identified, including ceramides (Cer), cholesterol esters and phosphocholines (PC). Examples from these lipid classes, show changes in expression over the time course (i.e., 3 to 24 hrs).

In particular, dysregulation of ceramides and cholesterol esters have been linked to oxidative stress, leading to induced abnormalities in inflammatory responses of microglia. Additionally, ceramides, which are the building blocks for sphingolipids are associated with neuronal bodies (grey matter). Increased PC levels have also been associated with acute and chronic phases of TBI due to excitotoxicity, ischemia and oxidative stress, therefore PC's could be potentially used for predicting the severity and prognosis of TBI's.



CONCLUSION

- **A novel study, indicating a positive effect on the recovery of TBI when treated with xenon gas. Lipidome established to be affected after TBI.**

⇒ **Lipidome in particular, showed alteration with TBI and distinct changes following xenon treatment.**

⇒ **Comparison of control gas and xenon highlighted differences in lipid expression.**

- **Integration of Data Convert into the waters_connect allows a seamless workflow of automatically generating mzML during data acquisition, in addition to having flexibility for users to export mzML from projects at any point in time.**
- **Datasets from the study, highlight the high quality data that can be generated from the Xevo MRT mass spectrometer:**

⇒ **Superior mass accuracy (sub 1 ppm)**

⇒ **High mass resolution and mass accuracy is maintained with fast, high throughput LC gradients**

⇒ **High levels of sensitivity/dynamic range**

References

1. Campos-Pires, R., *et al.,* Xenon Protects against Blast-Induced Traumatic Brain Injury in an In Vitro Model. *J Neurotrauma.* 15;35(**8**):1037-1044.
2. Pang, Z., *et al.,* MetaboAnalystR 4.0: a unified LC-MS workflow for global metabolomics. *Nature Comms.* (doi: 10.1038/s41467-024-48009-6).
3. Ikram, M., *et al.,* Melatonin as a Potential Regulator of Oxidative Stress, and Neuroinflammation: Mechanisms and Implications for the Management of Brain Injury-Induced Neurodegeneration. *Journal of Inflammation Research.* **14**, 6251-6264 (2021).

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