Ethanol-induced metabolomic differences in the Gut-Liver-Pancreas Axis

Simon Ashton[1]; Emily Armitage[1]; Neil J Loftus[1]; Olga Deda[2]; Christina Virgiliou[3]; Thomas Meikopoulos[3]; Matthew Harrison[3]; Ian D Wilson[4]; Helen Gika[2]

[1] Shimadzu MS/BU, Manchester, United Kingdom; [2] School of Medicine and CIRI BIOMIC_AUTh, Aristotle University, Thessaloniki, Greece; [3] Department of Chemistry and CIRI BIOMIC_AUTh, Aristotle University, Thessaloniki, Greece; [4] Imperial College London, London, United Kingdom

Overview

- Untargeted metabolomics utilising HRMS LC-MS/MS analysis has been applied to study the gut-liver-pancreas axis following ethanol exposure in mice.
- 8-week ethanol exposure resulted in tissue specific changes in metabolite and lipid profiles compared to the untreated control group.

1. Introduction

Excessive alcohol use is associated with neuropsychiatric disorders, cancers, cardiovascular disease, pancreatitis, and alcoholic liver disease. Although alcohol-induced disease is well characterized, the underlying pathology responsible for the development and progression of disease is poorly understood and few studies have considered the impact of ethanol-induced metabolomic changes in the gut-liver-pancreas axis. In this metabolomics study, high resolution mass spectrometry LC-MS/MS was used to measure changes in metabolite profiles in gut, liver and pancreas tissue samples following chronic exposure to ethanol in mice.

2. Methods

C57BL/6 Mice were subjected to chronic exposure to ethanol (8 un-dosed controls and 8 treated over an 8-week duration). Exposure to ethanol was achieved by feeding animals, ad libitum, with the Lieber-DeCarli ethanol diet, containing 5% extra pure ethanol. Tissues were collected post-mortem and, following tissue lysis and extraction, high resolution mass spectrometry LC-MS/MS (LCMS-9030 Shimadzu Corporation) was used for untargeted metabolite analysis. Data acquired precursor and product ion scanning spectra using data independent and data dependent acquisition methods using a mass range of m/z 100-1000. Data processing used LabSolutions Insight and MetaboAnalyst. The study was carried out in accordance with EU and National ethical guidelines and was approved by the Aristotle University of Thessaloniki.

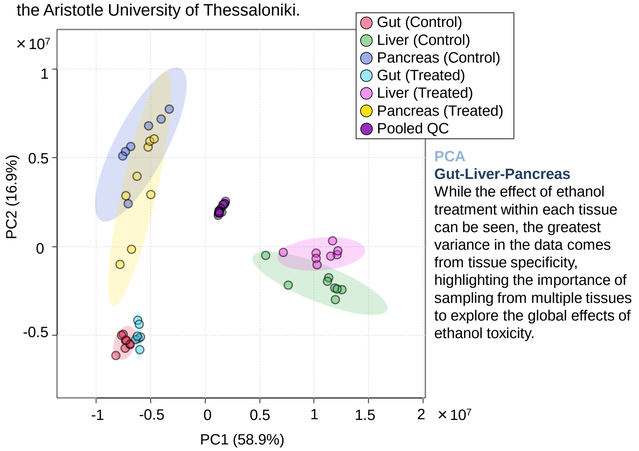

Figure 1. PCA scores plot for 2385 features extracted using HRMS LC-MS in positive ion mode with QC presence >50% and RSD<30%. MetaboAnalyst software was used to generate PCA and volcano plot analysis.

3. Results

Metabolic features were extracted from raw HRMS LC-MS data and filtered based on QC criteria (ion signals present in at least 50% of the QC samples with RSD <30%) for principal component analysis (Figure 1). Further statistical analysis included volcano plot analysis shown in Figure 2 (metabolic features present in at least 75% of either the control or treated group; fold change >2; p-value <0.05). The most significant features (FDR corrected p-values and log fold changes >2) that could be annotated are shown in Figure 3.

Figure 2. Volcano plots showing features extracted using HRMS LC-MS in positive and negative ion mode that were significantly increased or decreased in each tissue following ethanol administration (fold change >2, p<0.05).

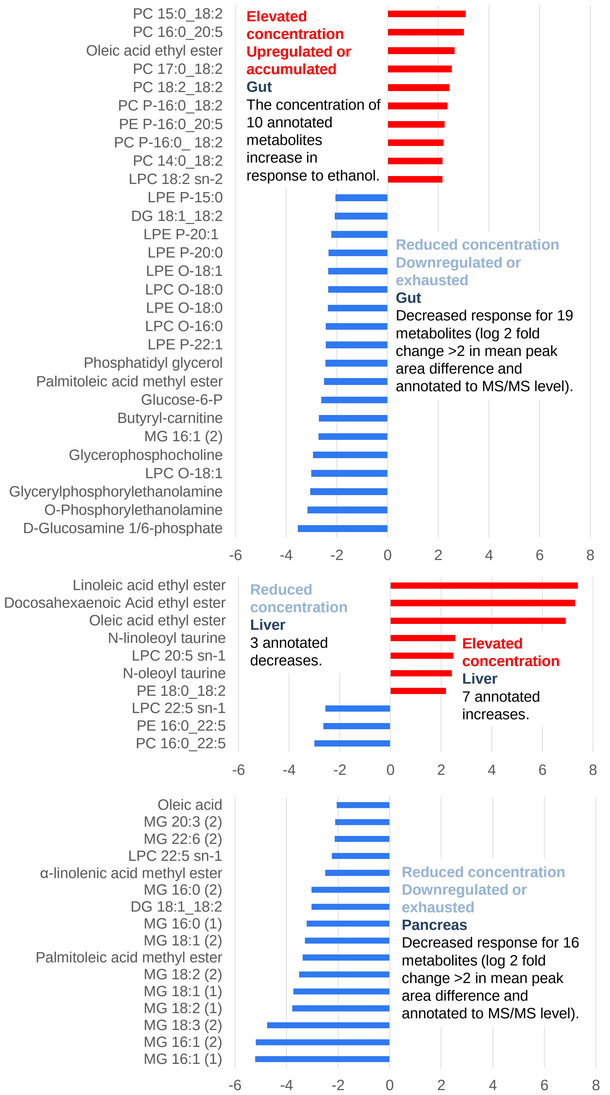

Figure 3. Bar-charts highlighting the most significant changes (log fold change >2) caused by ethanol administration in each tissue that could be annotated at the MSMS level (Metabolomics Standards Initiative level 2).

4. Discussion

- Data processing workflow
  - Precursor detection in the TOF MS mass scan using Analyze component detection algorithm (threshold set to low).
  - Pooled QC filters applied to the detected precursors.
  - Statistical analysis using MetaboAnalyst for PCA and volcano plots to find metabolites features with fold change >2; p-value <0.05. FDR correction was applied to select the most significant features with log fold change >2 for annotation.
  - Metabolite annotation used precursor and product ion scanning spectra with data independent and data dependent acquisition methods. The MS TOF mass scan used a mass range of m/z 100-1000; MS/MS data was acquired from m/z 40-1000 by reference to in house MS/MS libraries and external data repositories (Metlin, MassBank).
- Chronic exposure to ethanol resulted in the following changes
  - In the gut, a higher number of metabolite features changed compared to the liver or pancreas tissue extracts.
    - Notable increases included phospholipids and oleic acid ethyl ester.
    - Lyso-phospholipids (particularly alkyl and alkenyl linked forms), glycerophosphocholine and glycerophosphoethanolamine were decreased.
  - In the liver, fatty acid ethyl esters increased significantly with Log2 fold changes >6. Increases in N-acyltaurines were also observed, likely as a protective mechanism response.
  - In the pancreas, the most significant features were decreases in monoacylglycerols with log2 fold decreases between 2 and 6.
- Only 3 metabolites were common to gut-liver-pancreas tissues. Moreover, these were not the most significant changes in each tissue with Log2 FC<2 in most cases.
  - LPC 22:5 *sn*-1 and LPE 22:5 *sn*-1 decreased in all tissues
  - LPC 20:4 *sn*-2 decreased in the pancreas and liver and increased in the gut.

5. Conclusions

- A HRMS LC-MS/MS method was applied to study the ethanol-induced metabolomic differences in the gut-liver-pancreas axis. Significant changes in metabolite response were identified which were highly specific to each tissue.
- Marked increases in fatty acid ethyl esters were observed in the liver, while a panel of monoacylglycerols were reduced in the pancreas. Phospholipid increases were observed in the gut along with a reduction in lyso-phospholipids, glycerophosphocholine and glycerophosphoethanolamine.