Elucidation of metabolic changes in HFD-ApoE$^{-/-}$ model by SP6 peptide: A flow injection analysis MRMS study

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Introduction

Metabolic disorders, such as hypertension and dyslipidemia, are comorbid pathologic conditions often found in combination and characterized by a deep connection of altered molecular pathways. The employment of natural compounds in combination to pharmacological treatment can be an attractive option as preventive therapy. Natural peptides are among the most employed compounds. In this regard a novel antihypertensive decameric peptide has been recently characterized from the gastro intestinal digest of Spirulina platensis microalgae [1]. To understand the molecular pathways that are influenced by bioactive compounds, metabolomics has emerged as a leading approach. High resolution mass spectrometry (HRMS) is the workhorse for metabolomics applications, and MRMS due to its high mass accuracy and resolution, facilitates phenotyping studies. In this regard, the objective of this work was to investigate the effect of the SP6 peptide in a mice model of atherosclerosis (high fat diet ApoE$^{-/-}$) and evaluate the altered molecular pathways by a FIA-MRMS approach, termed MRMS aXelerate®. Results showed evidence of a distinct modulation of key molecular analytes involved in the disease development and open the way to further large-scale studies.

Methods

Animal treatment

ApoE$^{-/-}$ mice (Charles River Laboratories, Sant'Angelo Lodigiano, Italy) were randomly divided into the control group treated with saline solution and the group treated with peptide SP6 (5 mg/kg) by daily gavage administration. Mice were fed up to 10 weeks with normal rodent chow (4.5% fat; Ralston Purina Co.), and subsequently switched to the Western diet-high fat diet (HFD) (Complete feed for Rodents Purified Diet 60% ENERGY FROM FATS – Mucedola) at week 11 for 1 week. Subsequently, in the next week they were treated by gavage with saline solution (N = 4) or with SP6 (N = 5) daily for 4 weeks

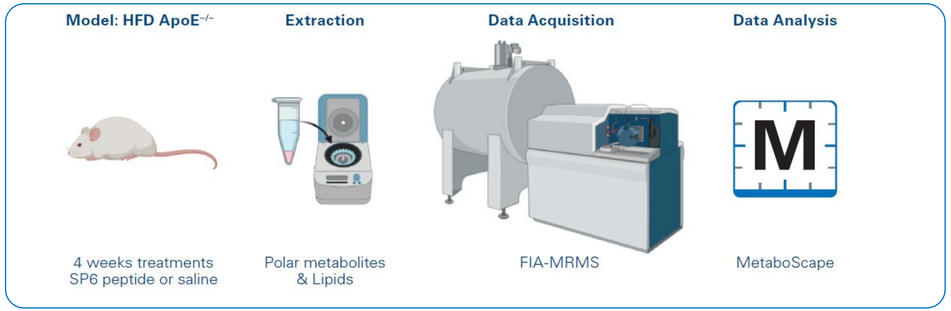

Figure 1: Workflow for the extraction and analysis of metabolites and lipids from high fat diet ApoE$^{-/-}$ mice atherosclerotic model.

At the end of treatment (4th week), blood was collected from the heart through cardiac puncture of isoflurane-anesthetized mice in heparinized tube, and rapidly centrifuged at 2200 rpm for 15 min to obtain plasma samples.

Sample preparation

Polar metabolites and lipids were extracted according to the Matyash protocol [2]. The workflow is shown in Figure 1. Mouse is sacrificed, plasma id obtained by centrifugation, analyzed by FIA-MRMS and data are processed with MetaboScape (Bruker).

FIA-MRMS analysis and processing

Ion source: TriVersa NanoMate (Advion)
Ion source parameters: voltage 1.45 kV, 5 µm nozzle
Mass spectrometer: solariX 7T XR
Mass range (lipids) : m/z 100 – 1500
Mass range (metabolites) : m/z 90-800
MS scans: 32 scans added with 2M data size
Data processing: MetaboScape 5.0 (Bruker)
Feature annotations: HMDB and LIPIDMAPS database with mass tolerance of 0.2 ppm (narrow) and 1 ppm (wide) and a mSigma value (accuracy of isotope pattern) below 200.

Results

The FIA-MRMS method was used for this study. The sample analysis time was less than 2 min including sample draw, analysis time and tip change. Mass accuracy values were on average 0.10 and 0.22 ppm for the detected polar metabolites and lipids respectively, which reflects the high mass accuracy of the MRMS platforms. This leads together with ultra-high mass resolution to unambiguous molecular formula assignment. The coefficient of variation (CV%) relative to peak intensity was between 0.11 and 10.95% for polar compounds (relative to the m/z range 100-750), and between 0.59 and 11.19% for lipids (relative to the m/z range 150-885) indicating a satisfactory repeatability for both metabolite classes. Data preprocessing was based on sample filter to remove peaks that were not present in at least 80% of a single group. Figures 2a and 2b show the results of PLS-DA model for plasma polar and lipids extracts, demonstrating that the two groups are clearly separated. Metabolites that contributed to the clustering and discrimination were extracted based on the variable importance in projection (VIP), which were generated after PLS-DA processing. The first 15 metabolites with highest VIP scoring of both polar and lipid extracts, are reported in Figure 2c and 2d. Different metabolite classes were found dysregulated, and in particular: hydroxyl and tricarboxylic-organic acids, amino acids, lysophosphatidylcholines, sphingomyelins, and other glycerophospholipids. In Table 1 statistical relevant Glycerophosphocholines as listed. The treatment with SP6 was able to modulate the levels of these key metabolites which are involved in atherosclerotic plaque progression and development.

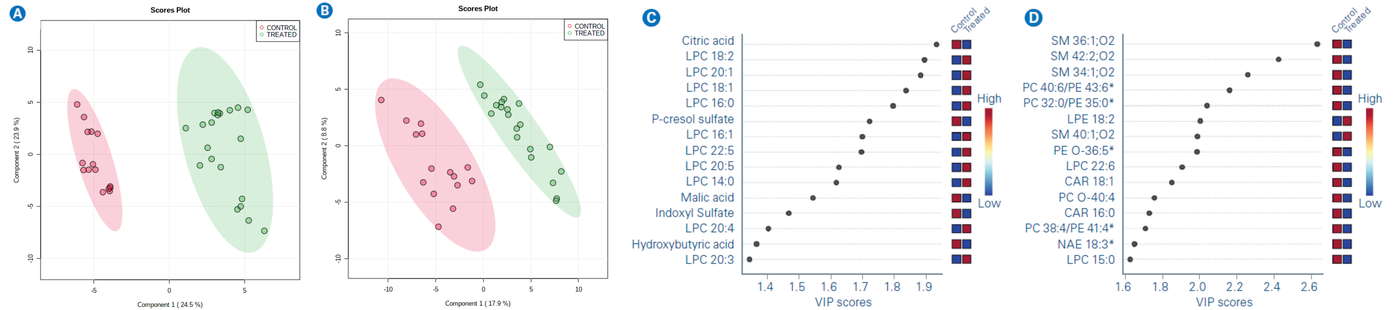

Figure 2: PLS-DA score plot of polar (A) and lipid (B) plasma extracts in HFD ApoE$^{-/-}$ mice treated with saline alone (red circle) and those treated with Spirulina peptide SP6 (green circle). The first 15 metabolites with the highest VIP scoring of both polar and lipid extracts, are shown in figure (C) and (D). Metabolite annotations displayed in 2C and 2D were confirmed by LC-MS/MS. * indicates compounds which MS/MS spectra were unavailable and/or cases in which molecular formula was shared among different metabolites. These were generally assigned to a class of compounds.

Table 1: Summary of statistical relevant annotated Glycerophosphocholines derived from PLS-DA analysis.

m/z	Ion type/ adduct	Assign-ments	Molecular Formula	Mass error in ppm	VIP value	p.value
482.32422	[M+H]$^+$	LPC 15:0	$C_{23}H_{48}NO_7P$	0.19	1.44	1.23E-02
490.29033	[M+Na]$^+$	LPC 14:0	$C_{22}H_{46}NO_7P$	0.12	1.6	8.90E-04
516.30599	[M+Na]$^+$	LPC 16:1	$C_{24}H_{48}NO_7P$	-0.07	1.68	1.80E-03
518.32162	[M+Na]$^+$	LPC 16:0	$C_{24}H_{50}NO_7P$	-0.14	1.78	2.10E-03
542.32164	[M+Na]$^+$	LPC 18:2	$C_{26}H_{50}NO_7P$	-0.54	1.87	3.60E-03
544.33724	[M+Na]$^+$	LPC 18:1	$C_{26}H_{52}NO_7P$	-0.59	1.81	4.70E-03
564.30598	[M+Na]$^+$	LPC 20:5	$C_{28}H_{48}NO_7P$	0.17	1.6	1.00E-02

Conclusions

- Peptide SP6 was initially evaluated in a model of atherosclerosis.
- MRMS aXelerate® was used for untargeted metabolomics and lipidomics.
- MRMS aXelerate® workflow delivers high mass accuracy, resolution, repeatability and fast analysis time.
- The modulation of key markers of atherosclerosis progression could be demonstrated.
- The results open the way to a large-scale study of SP6 preventive treatment.

MRMS Metabolomics

References
[1] Carrizzo et al. (2019), https://www.ahajournals.org/doi/10.1161/HYPERTENSIONAHA.118.11801
[2] Matyash et al. (2008), https://dx.doi.org/10.1194%2Fjlr.D700041-JLR200