Sphingolipid variations between hypertensive and normotensive patients elucidated by MRMS

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Introduction

Hypertension is one of the major worldwide causes of death. Although the precise cause-effect relationship is controversial, many studies have proposed that endothelial dysfunction may contribute to emergence of hypertension [1]. Sphingolipids are involved in the regulation of both vascular growth and vascular tone. Several reports have shown that in hypertension essentially sphingolipids levels are altered. The profiling of sphingolipids is usually carried out by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). As an alternative to LC-MS/MS, which suffers from long analysis time and lack of reproducibility due to retention time drift, direct infusion magnetic resonance mass spectrometry (DI-MRMS) provides short analysis time for sample screening including high mass accuracy as well as isotopic fine structure for metabolite identification, allowing analysis without the front-end separation step and derivatization. This MRMS workflow maximizes high throughput in metabolic profiling. In this study sphingolipid levels among healthy and hypertensive patients measured by of DI-MRMS were investigated.

Methods

Extraction

Twenty-four patients with hypertension (defined as DBP ≥90 mmHg and/or SBP ≥140 mmHg or on the basis of use of anti-hypertensive medication) and 9 healthy donor control subjects (non-smokers and non-diabetic) without previous cardiovascular events and not on statin therapy, belonging to the Campania Salute Network Registry, were studied. The database generation of the Campania Salute Network was approved by the Federico II University Hospital Ethic Committee. Signed informed consent was obtained from all the participants to use data for scientific purposes. Plasma samples were thawed and extracted with methanol (MeOH) and methyl-tert-butyl ether (MTBE). Briefly, 225 µL of MeOH was added to 20 µL of plasma and the mixture was vortexed for 10 seconds.

Then 750 µL of MTBE was added and the obtained solution was incubated at 300 rpm for six minutes at 4°C. Afterwards, 188 µL of H2O was added to induce phase separation and the mixture was vortexed for 20 seconds. After centrifuging for five minutes at 14680 rpm, 650 µL of the upper organic phase was transferred into a new vial and dried. For mass spectrometric analysis, the sample (organic phase) was solubilized in 200 µL of 5mM ammonium acetate 90%MeOH/DCM (2:1 v/v).

MS analysis

Analyses were performed by direct infusion (DI) using electrospray ionization (ESI) following a previous protocol using flow injection with 250 µL syringe at a flow rate of 2 µL/min.

Data were acquired on a solariX XR 7T (Bruker Daltonik GmbH, Bremen, Germany) MRMS system. The instrument was tuned and calibrated with a standard solution of NaTFA (0.1 mg/ml in 50% acetonitrile). Mass spectra were acquired in broadband mode in the range 100–1200 m/z with an ion accumulation time of 10 ms. 32 single scans were added for the final mass spectrum using 2 million data points (2M). Nebulizing (N2) and drying gases (N2) were set at 1 and 4 L/min, respectively, with a drying gas temperature of 200 °C.

Spectra were acquired in positive and negative ionization modes. To ensure good statistics and reproducibility five measurement replicates of each sample were performed.

Data processing

Peak alignment and tentative annotation of compounds based on accurate MS measurements were performed in MetaboScape 4.0 (Bruker Daltonik GmbH, Bremen, Germany). Adducts were combined and deisotoped during the feature generation.

LipidMAPS was used as the analyte list for compound identification. Comparisons and differences of samples and patient groups were analyzed by two-way Anova test and Bonferroni post tests analysis for statistical significance.

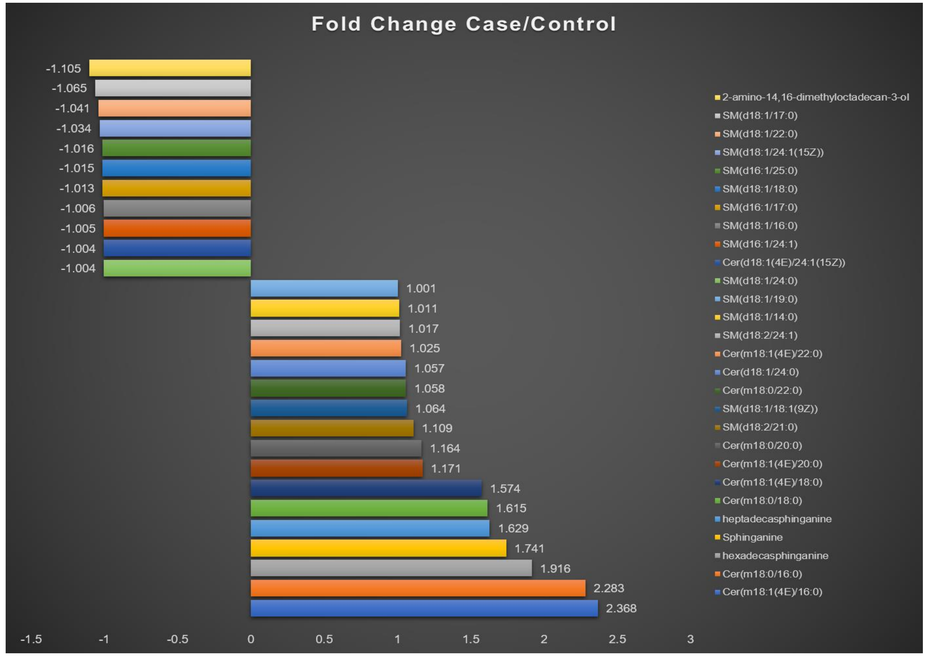

Figure 1: Fold change of mainly sphingolipids by hypertension - ratio of case (hypertensive) to control(normotensive) is shown

Results

Total lipid extracts from 33 human plasma samples were analysed in this study. The analysis of plasma lipid extracts showed a very complex profile. Roughly 200 lipids (considering both positive and negative ionization) were tentatively annotated, with very good mass accuracy (average error ≤ 0.1 ppm) and detected lipids belonging to different classes. Among them as shown in figure 1 different sphingolipids showed alteration between healthy and hypertensive subjects (patients).

This is shown in figure 1 reporting the fold change of mainly sphingolipids. Both groups, healthy and hypertensive, could be clearly separated in the statistic plot shown in figure 2. In particular the ceramide level, especially medium-length ceramides such as Cer (18:1(4E)/16:0) showed an increase of hypertensive patients. This data is supported by a growing number of publications of hypertensive studies [2].

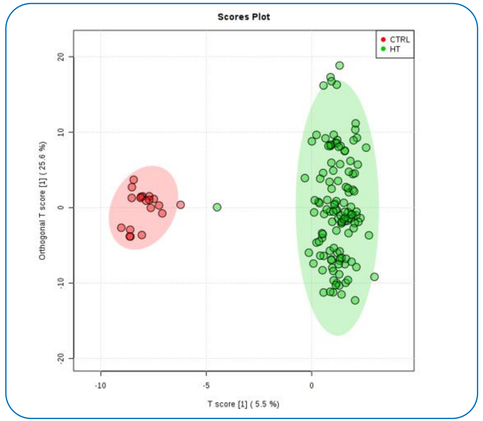

Figure 2: Both groups, healthy (CTRL, red) and hypertensive (HT, green), could be clearly separated with high significance in the statistic plot.

Conclusions

- DI-MRMS is a fast and reliable method for sphingolipids profiling.
- Several sphingolipids levels alter by hypertensive disease when comparing the healthy patients. These are in particular ceramides with medium length.
- This data further highlights the role of ceramides associated to endothelium disfunction

MRMS Lipidomics

References

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