Anti-inflammatory effects of hop bitter acids in dentritic cells revealed by MRMS metabolomic studies

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Introduction

Inflammation is a complex, evolutionarily conserved process, that involves immune and non-immune cells in the host for protection from harmful stimuli. Metabolic changes occur in macrophages and dendritic cells (DCs) leading to pro or anti-inflammatory phenotypes. In this regard, the growing field of immunometabolism aims to target specific metabolic pathways to modulate inflammation, and natural compounds are more and more used for preventive action [1]. To understand the metabolic shift toward anti-inflammatory phenotypes, metabolomics has emerged as leading approach. The metabolome profiling is usually carried out by liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).
As an alternative to LC-MS/MS, direct infusion magnetic resonance mass spectrometry (DI-MRMS) or flow injection (FIA) MRMS delivers short analysis time for sample screening and features unmatched mass accuracy, resolution, sensitivity, and isotopic fine structure. Hence, the FIA- or DI-MRMS workflows provide a higher throughput in metabolic profiling compared to LC-MS methods [2]. In this study, MRMS was used to highlight the metabolic changes in dendritic cells stimulated with lipopolysaccharide (LPS) and co-treated with a Hop derived fraction rich in beta acids and prenylflavonoids and compared their effect with the flavonol quercetin.

Methods

- NanoMate coupled to a solariX XR
- 32 scans added
- 2M data points
- Positive and negative ion mode
- Five replicates of each sample
- Processing with MetaboScape using HMDB and LipidMAPS
- Metaboanalyst 5.0 for PCA and PLS-DA statistical analysis

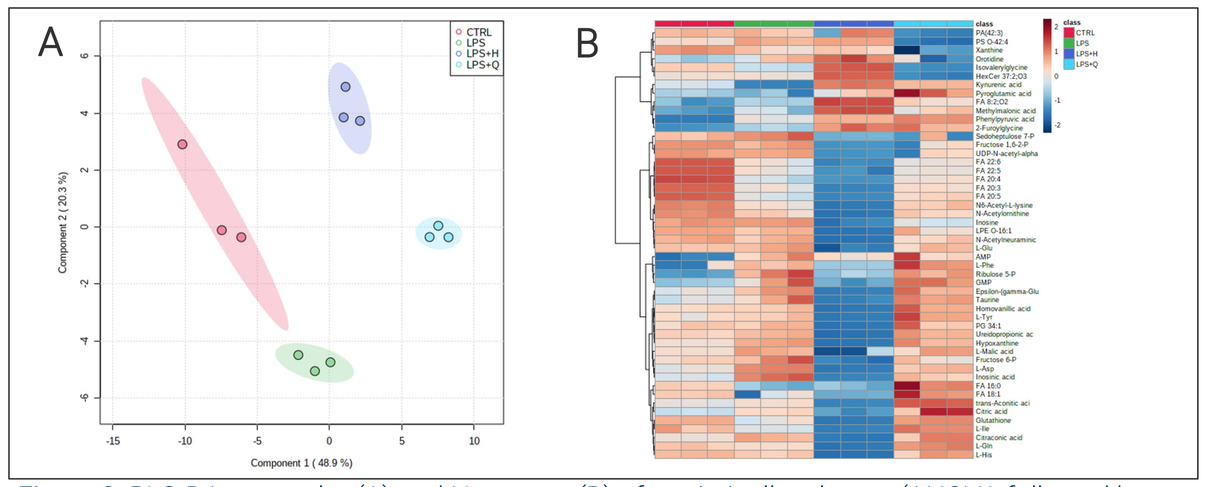

Figure 2: PLS-DA score plot (A) and Heat map (B) of statistically relevant (ANOVA followed by a Tukey's post hoc, $p < 0.05$) DCs metabolites modulated by LPS, HOP C, and quercetin. Color changes reflect normalized intensity.

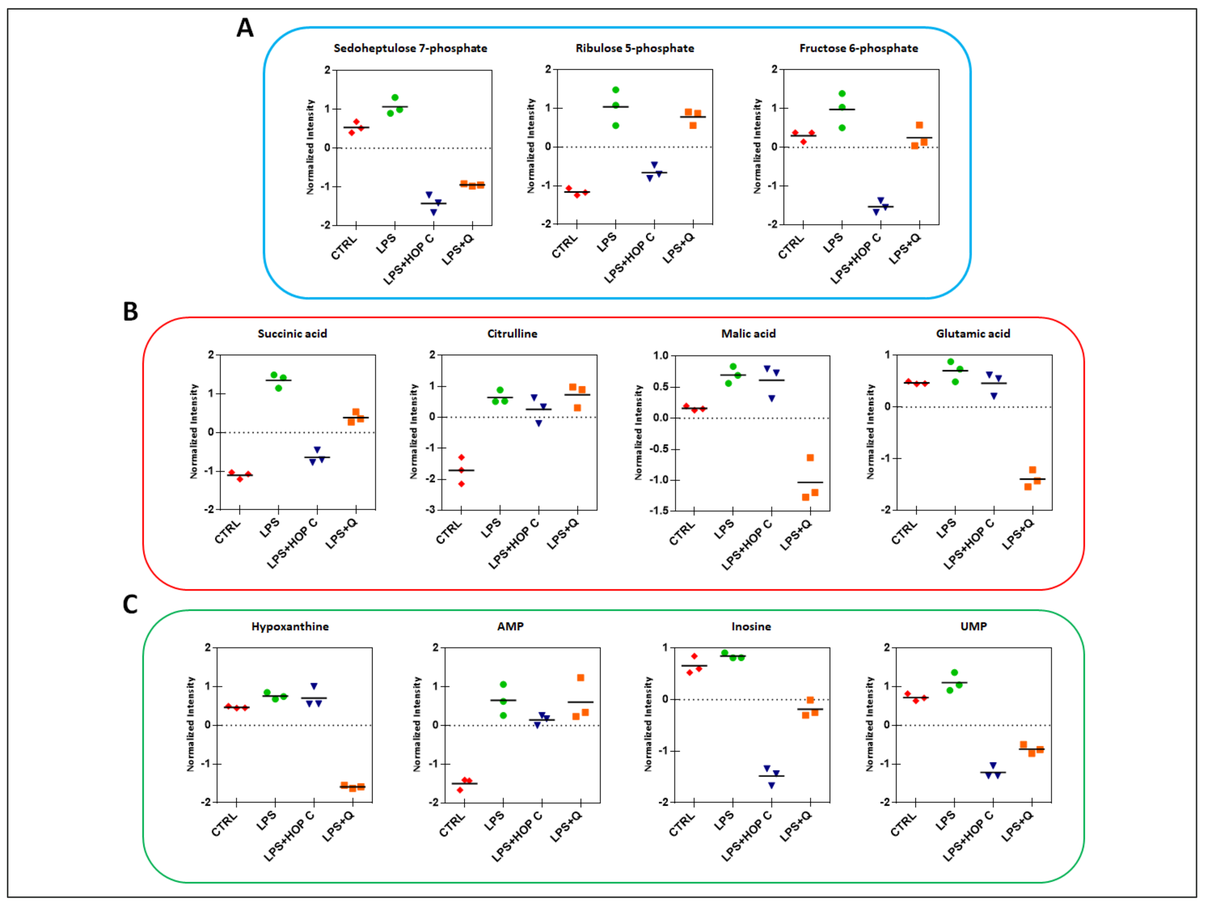

Figure 3: Normalized intensity of altered metabolites involved in metabolic reprogramming of LPS-DCs and modulated by HOP C and quercetin treatments: (A) Pentose phosphate pathway intermediates, (B) TCA cycle and citrulline, (C) Purine/Pyrimidines and Nucleotides.

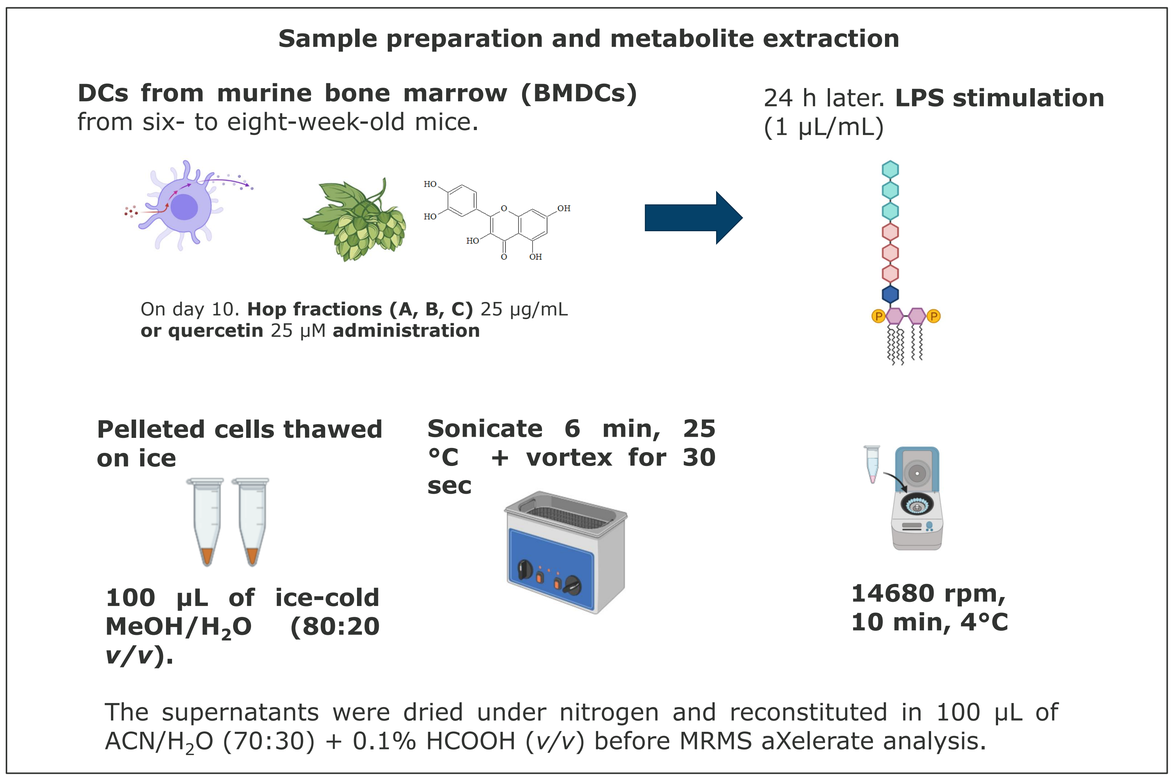

Figure 1a: Sample preparation and metabolite extraction.

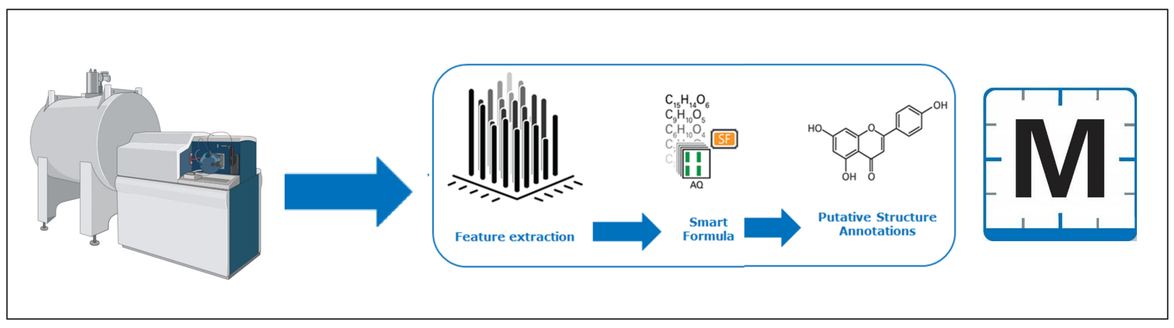

Figure 1b: MRMS aXelerate workflow using nESI.

References
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Results

DCs stimulated with LPS pre-exposed to 25 µg/mL of HOP B and C fractions showed reduced production of several inflammatory cytokines (IL-6, IL-1α, IL-1β and TNF). The fraction C was significantly more effective than the fraction B. Based on the results of inflammatory cytokine and qPCR measurements, the DCs samples were divided into four groups: control (CTRL), LPS stimulated DCs (LPS), LPS-DCs plus HOP C (LPS+H) and plus quercetin (LPS+Q). As can be observed in the PLS-DA score plots in Figure 2A, metabolites in the different DCs groups were separated into distinct clusters. The metabolite abundance variations induced by the LPS stimulation and after the two treatments were visualized by a heatmap shown in Figure 2B.

This untargeted metabolomics study showed that quercetin and HOP C induced changed are segregated in two independent pathways. LPS-activated DCs are characterized by anaerobic glycolysis and a pro-inflammatory phenotype [3]. As shown in the Whisker box plots in Figure 3, the preventive administration of HOP C acts through a regulation of the oxidative and non-oxidative branch of pentose phosphate pathway, regulates the arginine-citrulline and arginine-succinate shunt, and normalizes the nucleotide metabolism, differently from quercetin. These results suggest that HOP C fraction is effective to impair the inflammatory response by regulating the metabolic reprogramming of DCs toward anti-inflammatory and resting conditions.

Conclusions

- MRMS aXelerate is a fast and reliable method for metabolome profiling.
- MRMS aXelerate allows to take the snapshot of metabolic changes in BMDCs stimulated by LPS and co-treated with a Hop bitter acids and prenylflavonoids fraction.
- Highlights the potential role of natural compounds in targeting immunometabolism treatment of inflammatory diseases.

MRMS Metabolomics