Metabolic changes in murine hair follicles treated with procyanidin-B2 rich nutraceuticals by magnetic resonance mass spectrometry (MRMS)

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Introduction

Patterned hair loss affects around 50% of the adult population worldwide. Independent of age and gender, this condition exerts a profoundly negative impact on people's quality of life and is frequently associated with depression, especially when occurring at an early age. Among pharmacological treatments, Finasteride and Minoxidil are FDA approved drugs. However, Finasteride and Minoxidil activity reaches a plateau within two years of usage and both drugs produce adverse effects on patients. In the past decade an increasing number of reports have proven nutritional and antioxidant therapies to be an effective and safe treatment as an option for hair loss. Among these, nutraceuticals enriched in Procyanidin-B2 (Figure 1), a dimeric Procyanidin, such as Annurca apple extract (AAE), have been recently shown to promote hair growth and induce anagen phase in humans. This has resulted in the increased usage of these type of nutraceuticals for hair growth over the last several years. However, studies reveal this mechanism is far from being complete. Here, the metabolomic profile of AAE treatment on mice hair follicles was studied to give new insights into the promotional growth effects of Procyanidin-B2 (figure 2).

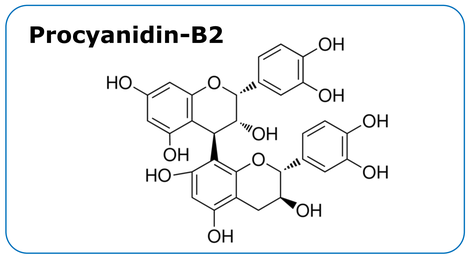

Fig. 1: Structure of Procyanidin-B2, a B type proanthocyanidin - (−)-Epicatechin-(4β→8)-(−)-epicatechin

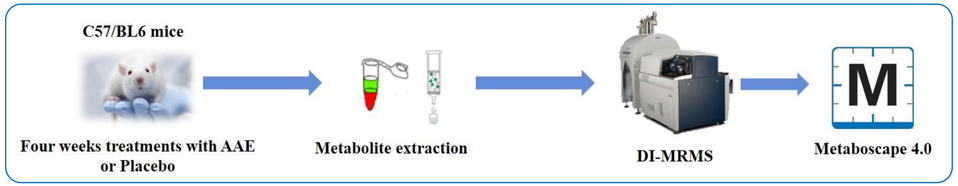

Fig. 2: Schematic workflow: a) Treatment of mice with Annurca apple extract (AAE) or placeto, b) metabolite extraction, c) detection of metabolites with direct infusion MRMS, d) analysis of mass spectrometric data with MetaboScape 4.0 using HMDB plasma analyte list.

Methods

Samples:

Wild-type C57/BL6 mice (7 weeks old, postnatal day 42) were used in all experiments to test the effect of cosmetic foam containing Annurca apple extract (AAE). All animals received humane care and were maintained in separate cages at 22 C – 24 C and fed a general rodent diet. Animals were left unshaved and topically treated with 2 cm^3 of the indicated cosmetic foam for 4 weeks, twice a week. Mice tissues were rinsed and kept in PBS immediately after tissue excision. Hair shafts were plunked and covered with a solution of PBS at room temperature. Plunked hair shafts were incubated for 15 minutes in PBS supplemented with 5 mM EDTA. Hair shafts were removed while hair follicle cells. Cell pellets were washed twice in PBS and homogenized in 1 ml of pre-chilled methanol/water 80:20 solution.

Data Acquisition:

Analyses were performed by direct infusion ESI. Data were acquired on a solariX XR 7T MRMS. Mass Spectra were recorded in broadband mode in the range m/z 100 - 1500. Spectra were acquired in positive and negative ion mode. The measurements were performed in five replicates (Figure 2).

Data processing

Peak alignment and tentative assignment of compounds was performed in MetaboScape 4.0 based on accurate mass and HMDB plasma analyte list. The feature calculation was performed with a mass resolution of 1 mDa. A bucket filter of 75% was used for replicate measurements and the values of the calculated features were recalibrated with accurate masses of compounds known in plasma. Accuracy of isotopic peaks with a maximum mSigma value of 50 was used for feature assignment. Statistical analysis with significant results were performed with Statistica® using two-way Anova and Bonferroni post tests.

Results

The workflow using direct infusion MRMS and MetaboScape is shown in Figure 2. The ultra-high mass accuracy (average mass error of only 0.166 ppm, Table 1), exact isotopic distribution and comparison with available standards ensured identification with high confidence. By screening intracellular metabolites with similar alteration tendency in all the AAE treated mice, glutaminolysis, pentose phosphate pathway (PPP), amino acid phosphate pathway (PPP), amino acid oxidation, mitochondrial β-oxidation as well as Arginine metabolites became our focus. Significant elevation of glutamine and glycine as well as the increase in the intra-cellular level of the PPP intermediate Ribulose 5P together with the reduction of the intracellular level of nucleotides and deoxy-nucleotides suggest that AAE cause a reduction in the utilization of glucose and gluta-mine for PPP (Figure 3). This is a metabolic pathway that correlates with nucleotide biosynthesis in hair follicles. The reduced intracellular level of glutathione also confirmed that the catabolism of glutamine is halted in AAE treated hair follicles. Statistical analysis supports these results (Figure 4). This verifies the observed results of the regulation of the metabolites shown in table 1. From the metabolite profiles it can be concluded that Annurca apple extract containing Procyanidin-B2mdiverts the intracellular metabolism of hair follicles from mainly set on PPP to a pool of selected amino acids to be used for keratin biosynthesis. Overall, considering the results of SEM data (not shown here) and metabolite profiles we suggest that AAE diverts the intracellular metabolism of hair follicles from mainly set on PPP to a pool of selected amino acids to be used for keratin biosynthesis.

Table 1: List of relevant metabolites for treatment of hair follicles with apple Annurca foam detected by DI-MRMS

Metabolite	Pathway	m/z	Detected as	Mass error (ppm)
Glucose	Glycolysis	203.05265	[M+Na]+	0.006
Lactic acid		113.02091	[M+Na]+	-0.176
Maltose	Glycogenolysis	365.10543	[M+Na]+	-0.012
Glutamine	Aminoacids	169.05836	[M+Na]+	0.001
Arginine		197.10090	[M+Na]+	0.029
Glutathione		306.07675	[M-H]-	0.028
Citrulline		198.08495	[M+Na]+	0.072
Adenosine	Nucleotides	290.08596	[M+Na]+	0.028
Cytosine		266.07476	[M+Na]+	0.015
Deoxy-Cytosine		250.07984	[M+Na]+	0.044
Deoxy-Inosine		275.07507	[M+Na]+	-0.091
Palmitoyl-carnitine	b-oxidation	422.32404	[M+Na]+	0.148
Acetyl-carnitine		226.10501	[M+Na]+	0.211

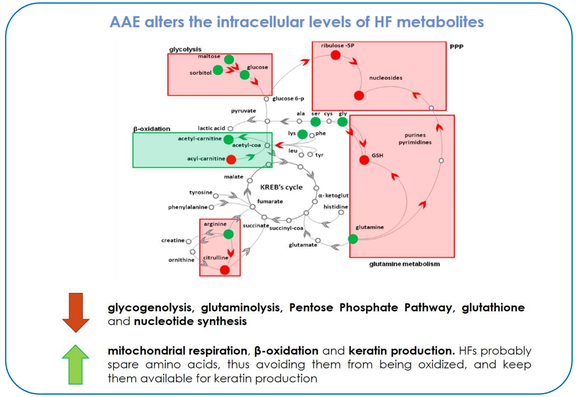

Fig. 3: Modulation of mice Hair follicles metabolites following treatment with apple Annurca foam

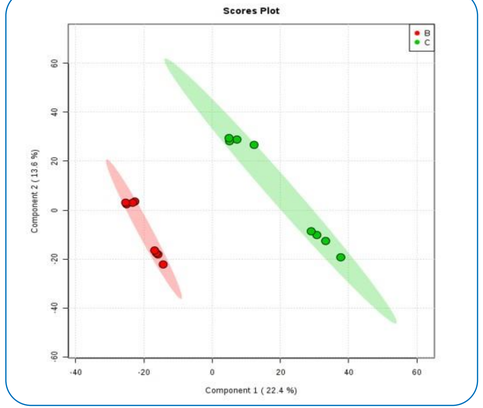

Fig. 4: PLS-DA scores plot of control hair follicle (red) vs treated (green) with apple Annurca foam

Conclusions

- Direct infusion MRMS can be used for fast and reliable metabolomic profiling of hair follicle cells treated with Annurca apple extract (AAE).
- Several metabolites involved in different metabolic pathways could be detected and identified by MRMS.
- Metabolic shift of hair follicle cells towards production of keratin was elucidated.

MRMS Metabolomics