Impact of instrument, detection method, and statistical methods in mouse plasma metabolite profiling

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Abstract

Changes in the metabolomic profile of plasma reflect the metabolic state of an organism and may reveal disease biomarkers or depict the phenotype of an animal model. For a thorough study of the said changes, reproducible and, more so, fast methods for high-throughput non-targeted screening of plasma are essential. The aim of this study was the comparison of mass spectrometric plasma metabolite measurements among a group of wild-type and KsiK knock- out mice across three methods; ultra-high resolution instrument (scimac MRMS 7T) coupled with a) ESI using flow injection analysis, b) MALDI and c) MALDI-TOF-MS.

Mass spectrometric results were analysed by Metaboanalyst statistical toolkit (Metaboanalyzer 4.0, McGill University, Quebec, Canada). Number of discriminative peaks identified by each instrument and number of suggested enzymes and pathways were compared. In negative ion mode, the ultra-high resolution instrument (scimac MRMS 7T, Bruker Daltonics Inc.) detected the highest number of peaks (5964 by ESI-MRMS and 6728 by MALDI-MRMS) compared to 520 peaks detected with a MALDI-TOF instrument (rapifleX, Bruker Daltonics Inc.). Statistical analysis by t-test revealed highly different number of discriminative peaks between MRMS and TOF masses (fold change > 2 with FDR score < 0.05; 1067 by ESI-MRMS, 930 by MALDI-MRMS compared to only 53 peaks by MALDI-TOF). With every method we were able to putatively annotate hundreds of peaks and thus depict enrichment in particular number of metabolic pathways, namely with data obtained with MRMS instrument (34 pathway hits with MALDI-MRMS and 20 hits by ESI-MRMS). Nevertheless, only the MRMS instrument showed significantly higher number of metabolites to depict changes in the metabolome.

Consequently, ultra-high mass resolution flow injection analysis can be used for fast metabolite profiling of plasma.

Materials and Methods

Plasma withdrawal

KsiK-deficient (n=5) and littermate wildtype (n=5) mice were anaesthetised and plasma was withdrawn into heparinised tubes (preparation of plasma was collected into aliquots and stored at -80°C).

For plasma was diluted 1000:1 with methanol and precipitated at -20°C, supernatant was further diluted 3 times with methanol and 20 µl premade 20 µl maleic acid and 10 µl 1 % acetic acid was added to 20 µl premade 20 µl 0.5 % acetic acid to the plasma sample. The mixture was then dried down and reconstituted with 10 µl of 95% methanol (v/v) and matrix (2 µl solution). 20 µl ESI 20 µl MALDI and 20 µl TOF TOF needles were used for matrix-assisted laser desorption in negative ion mode. Three technical replicates were evaluated in the study for each animal (n=5).

Sample Preparation and Data Acquisition Time

Sample preparation took 30 min, data acquisition took 35 min, in total 105 min, 30 µl of plasma per sample.

Results

Flow injection analysis with mass spectrometric identification of known and unknown compounds in plasma samples.

Acknowledgement

We would greatly acknowledge Dr. Matthias Witt and the Bruker Daltonik GmbH, Bremen, Germany, for the possibility of measurement of plasma samples with scimac MRMS 7T instrument.