Metabolic Profiling in Plasma Patients Administered with Tranexamic Acid

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ABSTRACT

Metabolic fingerprinting is a powerful tool in bioclinic. It allows for better understanding of mechanisms of physiological and pathological reactions. Analysis of clinical samples provides not only information about individual response on the treatment used but also about the medical condition of the patient. Although metabolomics is still gaining importance in the scientific field, sample preparation is still the main problematic step in entire process of metabolomics analysis. The main objective of the present study was to determine the metabolic profile of patients undergoing surgery with the use of cardiology bypass and concurrent administration of tranexamic acid. For the first time, direct immersion solid phase microextraction technique (Di-SPME) was introduced for metabolomics studies in human clinical plasma samples. Compounds in the extracts were further identified and quantified with LC-MS Exactive Orbitrap platform. Multivariate principal component analysis (PCA) was performed to find variables contributing to responses caused by treatment used. The results obtained showed changes of diverse compounds involved in biochemical pathways induced by the use of CPA and the drug administered. Moreover, individual differences in response to treatment were reported. Our studies showed that solvent-free direct extraction from plasma enables us to obtain a wide range of compounds that vary in chemical and physical properties. This indicates that Di-SPME coupled with LC-MS analysis can be further used for studies of human metabolome.

INTRODUCTION

For the last several years—omics analysis has been successfully applied in drug metabolism studies, clinical diagnostics, and toxicology. Although metabolomics has rapidly expanded over the recent years, its applications and new developments have been reported, sample preparation is still the most problematic step in the entire process of metabolomics analysis. Sample-preparation protocols using conventional techniques, e.g. liquid-liquid extraction (LLE) or solid-phase extraction (SPE), remove proteins and other biological matrixes from biological samples. However, these methods do not provide appropriate sample clean-up, therefore method matrix can occur when MS detection is used. Moreover, in case of LLE analysis of polar and non-polar analytes are performed from two different fractions, what increases the number of samples and overall time of analysis. SPE is rather used for targeted metabolomics due to its selectivity. Solid phase microextraction (SPME) is sample preparation method based on diffusion of free analyte to extraction phase. For global metabolomics SPME is mostly used for volatile compounds, while its application for plasma analysis was focused on specific analytes. In this work we present SPME protocol providing restricted access to large biomolecules such as proteins or phospholipids, which also contribute to matrix effect when MS analysis are performed.

BACKGROUND ANO OBJECTIVE OF THE STUDY

Excessive bleeding after cardiology bypass operation (CPB) is one of the most common complications of cardiac surgery. Tranexamic acid (TXA) is a foreward antifibrinolytic agent used in cardiac surgery. It competitively inhibits the activation of plasminogen to plasmin and directly inhibits plasmin activity at much higher doses. It was also reported that Lys analogues, such as tranexamic acid, promote proinflammatory and proatherosclerotic lipoprotein(a) (LP(a)) assembly in vitro. On the basis of in vitro studies with transgenic mice, it was hypothesized that Lys analogues increase plasma LP(a) levels by increasing the dissociation of cell-bound apheresin in combination with unidentified apheresinII. The objective of our study was to employ SPME for human metabolome analysis in patients undergoing cardiac surgery with the use of cardiology bypass. In addition to standard anesthesia, patients received TXA to prevent excessive bleeding. Direct extraction from plasma with the use of SPME-mix probe was followed by liquid chromatography separation and analysis on Exactive Orbitrap Mass Spectrometer.

EXPERIMENTAL WORKFLOW

EXTRACTION

EXTRACTION 300L, 500L or 1000L B-acetaminohexanoic acid (200 uL, v/v) LC-Resovon LC Positive mode

RESULTS OF PRINCIPAL COMPONENT ANALYSIS

Cardiac surgery — induces the reduction in circulating LDL, it is the movement of LDL to the extracellular matrix in U.S. Lp(a) — a product of oxidative modification of low-density lipoprotein (LDL) — TA — influence dyslipidemia mediated by Lp(a) leading with LDL and BPL.

TENTATIVE IDENTIFICATION OF THE COMPOUNDS

Human Metabolome Database + Simulation Spectra

ANALYSIS OF OUTLIERS

Individual response on therapy complicating the condition of the patient

Table. Method validation: average, standard deviation (SD) and relative standard deviation (RSD) calculated for three chosen m/z features in QC samples.

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The criteria of acceptance for metabolic profiling are still not defined. However, FDA recommendation for biomarkers regarding relative standard deviation is 30% of total error for targeted LC-MS analysis.

ACKNOWLEDGMENT

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CONCLUSIONS

• Di-SPME coupled with LC-MS platform can be successfully used for studies of human metabolic fingerprint
• with the use of new extraction phase it is possible to extract compound with different chemical and physical properties without any prior sample preparation step directly from plasma aliquot
• obtained results need further research about metabolites which are biologically active (free) and are able to interact with receptors
• metabolome profile can give a various information about changes in biochemic pathways induced by drug(s) administration and the used medical procedures
• analysis of outliers may provide additional insight into individual response on treatment employed or medical condition of the patient

References: