Metabolic Profiling in Plasma Patients Administered with Tranexamic Acid

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ABSTRACT

Metabolic fingerprinting is a powerful tool in bioscience. 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 metabolome analysis. The main objective of the present study was to determine the metabolic profile of patients undergoing surgery with the use of cardiopulmonary 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 response on the treatment used. The results obtained showed changes of diverse compounds involved in biochemical pathways induced by the use of CPB and the drugs 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 few years '–omics' analysis have been successfully applied in drug metabolism studies[1], clinical diagnostics, and toxicology[2]. Although metabolomics has rapidly spread out around the scientific field and its new applications have been reported, sample preparation is still the most problematic step in the entire process of metabolome analysis. Sample-preparation protocols using conventional techniques, e.g. liquid-liquid extraction (LLE) or solid-phase extraction (SPE), remove proteins and other biological molecules from biological samples. However, these methods do not provide appropriate sample clean-up, therefore matrix effect 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 not global metabolomics due to its selectivity[3].

Solid phase microextraction (SPME) is sample preparation method based on diffusion of free analyte to extraction phase[4]. For global metabolomics SPME is mostly used for volatile compounds, while its application for plasma analysis was focused on pharmacokinetics[5] and ligand-protein studies[6-8]. This arises from the fact that there are a limited number of commercially available coatings suitable for extraction from complex biological samples. The use of new mix-mode coatings allows for simultaneous extraction of hydrophobic and hydrophilic compounds. The sample preparation step involves only direct extraction from plasma (or other biofluid) followed by desorption in an appropriate solvent. Simplicity of the procedure allows one to avoid the loss of analyte as well as their chemical modification during sample preparation. Solvent-free extraction ensures the lack of dilution, which is an extremely important feature considering the low concentrations of most of endogenous compounds. Since the volume of extraction phase is very small and the nature of SPME is non-exhaustive extraction, the amount of extracted analyte is very small. However, this requires application of a sensitive MS instrument for detection. This feature also has an advantage over standard extraction techniques since the amount of co-extracted compounds causing ion suppression is minimal. Biocompatibility of SPME probes used for the aforementioned studies provide restricted access to large biomolecules such as proteins or phospholipids, which also contribute to matrix effect when MS analysis are performed.

BACKGROUND ANS OBJECTIVE OF THE STUDY

Excessive bleeding after cardiopulmonary bypass (CPB) is one of the most common complications of cardiac surgery[9-11]. Tranexamic acid (TXA) is a forefront 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, prevent prothrombotic and proatherogenic lipoprotein(a) (Lp(a)) assembly *in vitro*[12]. On the basis of *in vivo* studies with transgenic mice, it was hypothesized that Lys analogues increase plasma Lp(a) levels by increasing the dissociation of cell-bound apo(a) in combination with reducing Lp(a) catabolism[13].

The objective of our study was to employ SPME for human metabolome analysis in patients undergoing cardiac surgery with the use of cardiopulmonary bypass. In addition to standard anesthesia, patients received TXA to prevent excessive bleeding. Direct extraction from plasma with the use of SPME mix-mode probes was followed by liquid chromatography separation and analysis on Exactive Orbitrap Mass Spectrometer.

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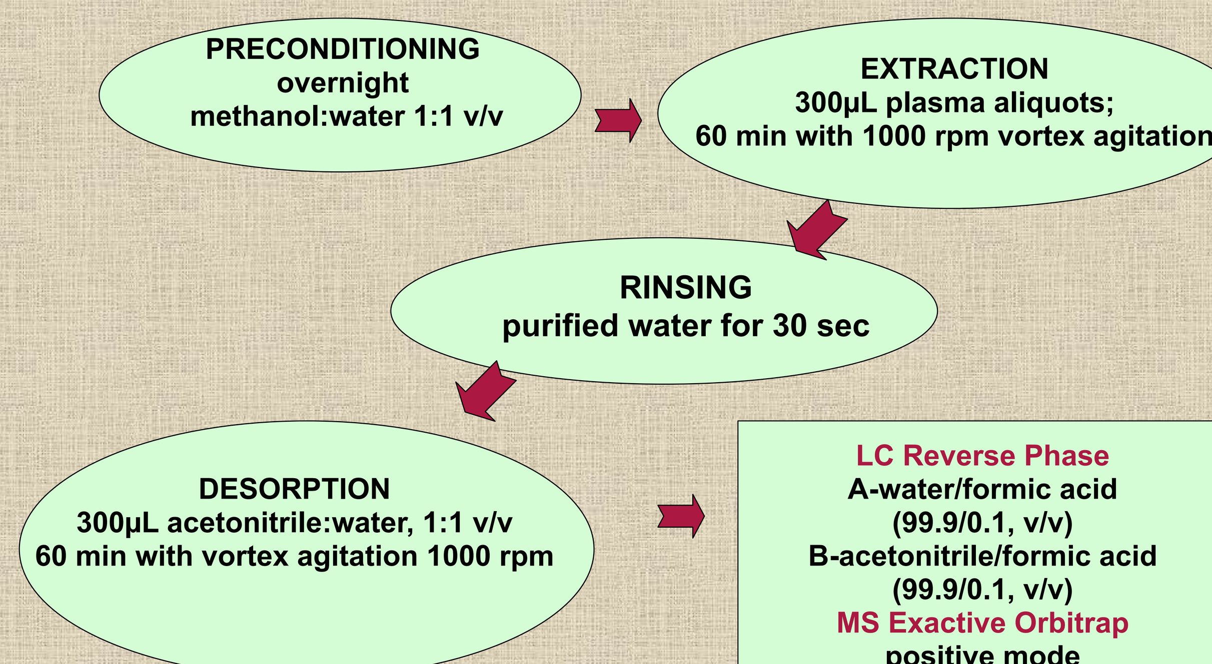
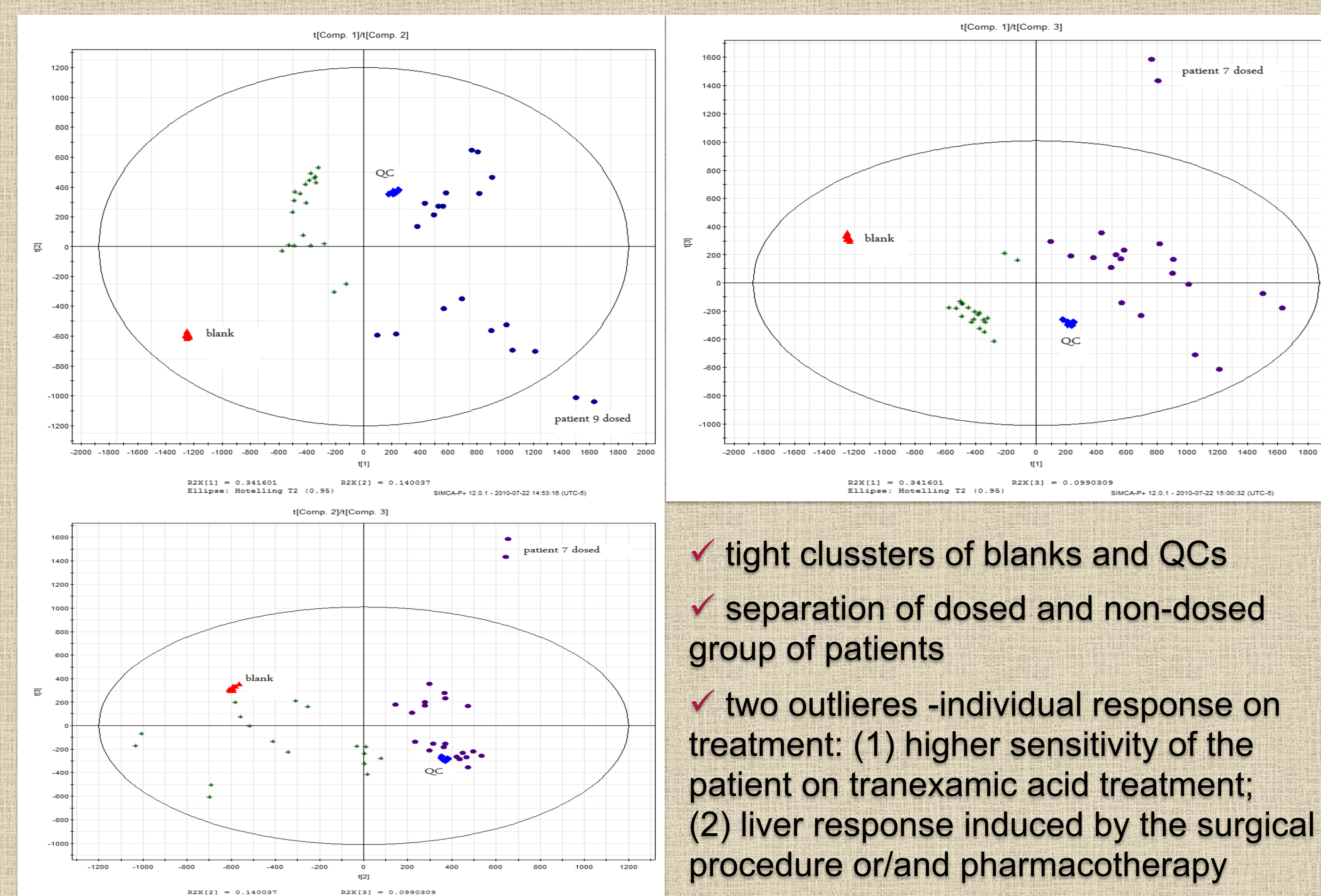
EXPERIMENTAL WORKFLOW



RESULTS OF PRINCIPAL COMPONENT ANALYSIS



✓ tight clussters of blanks and QCs

✓ separation of dosed and non-dosed group of patients

✓ two outlieres -individual response on treatment: (1) higher sensitivity of the patient on tranexamic acid treatment; (2) liver response induced by the surgical procedure or/and pharmacotherapy

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

	m/z	intensity	RT	m/z	intensity	RT	m/z	intensity	RT
avg	120.0657	82266.67	1.41	391.2840	12300.00	24.89	714.4723	2233.33	21.59
SD		9500.18	0.01		300.00	0.01		265.02	0.01
RSD		11.55%	0.41%		2.44%	0.04%		11.87%	0.03%

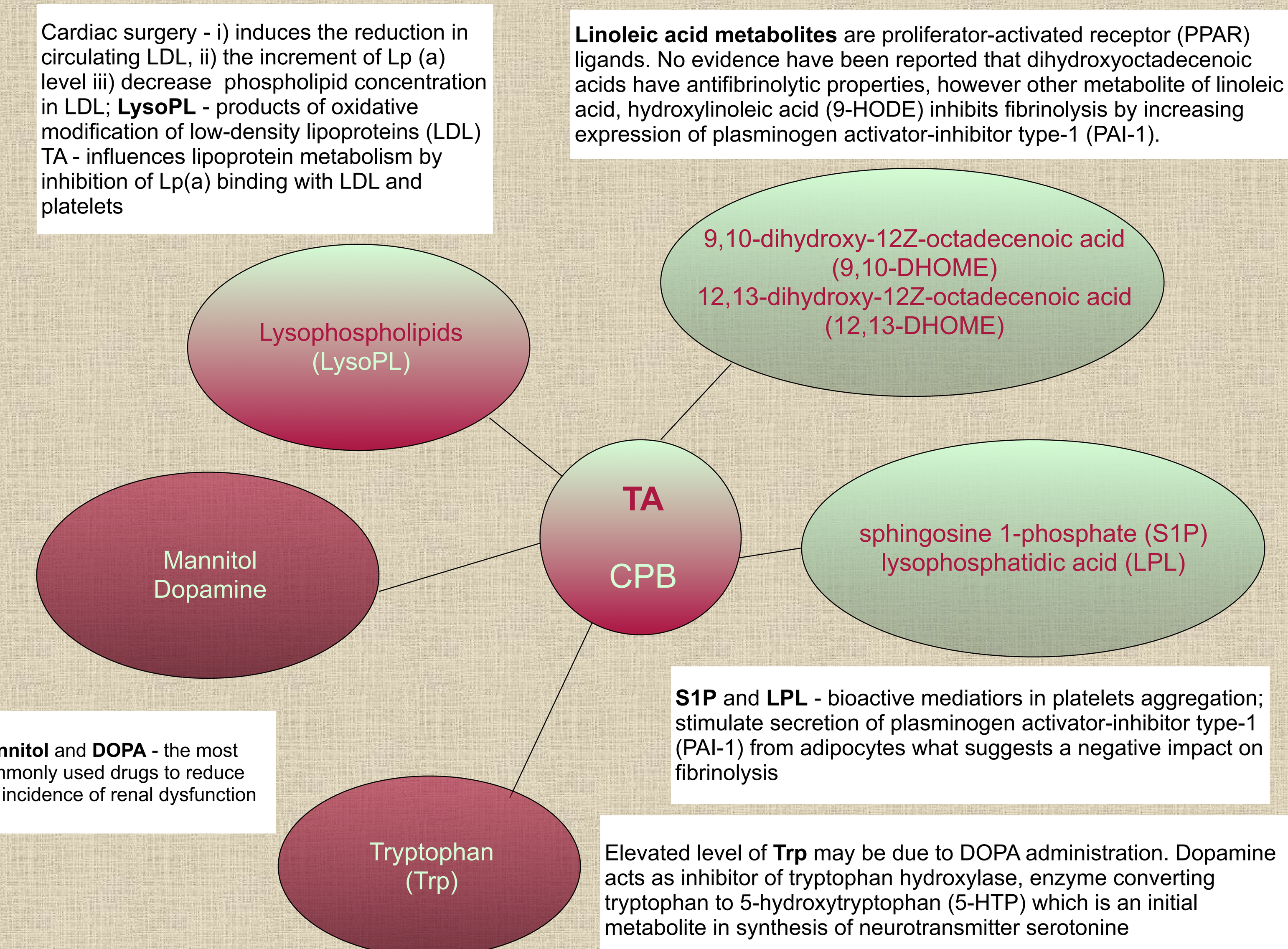
The criteria of acceptance for metabolic profiling are still not defined. However, FDA recommendation for biomarkers regarding relative standard deviation is 30% of total error for targeted LC-MS analysis.

ACKNOWLEDGMENT

Authors would like to greatly aknowledge Supelco for providing biocompatibile mix-mode fibres for the studies

TENTATIVE IDENTIFICATION OF THE COMPOUNDS

Human Metabolome Database + Simulation Spectra



ANALYSIS OF OUTLIERS

Individual response on therapy/clinical condition of the patient

PATIENT 7	PATIENT 9
Bile acids (tentative acids):	Linoleic acid and its metabolites (tentative)
•Chenodeoxycholic acid glycine conjugate	•HOME
•Deoxycholic acid glycine conjugate	•EpODE
•Chenodeoxyglycocholic acid	•HOTE
•Hyocholic acid	•EpODE
•Cholic acid	•OxoODE
•6a,12a-Dihydroxylithocholic acid	•DiHODE
•Allocholic acid	•HPODE
•Ursocholic acid	Eicosapentanoic acid
•Muricholic acid	Lysophospholipids

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 aliquots

✓ obtained results provide information about metabolites which are biologically active (free) and are able to interact with receptors

✓ metabolome profile can give a various information about changes in biochemical 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