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ORIGINAL ARTICLE

Metabolic profiling of plasma from cardiac surgical patients concurrently administered with tranexamic acid: DI-SPME–LC–MS analysis

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Abstract A metabolic profile of plasma samples from patients undergoing heart surgery with the use of cardiopulmonary bypass (CPB) and concurrent administration of tranexamic acid was determined. Direct immersion solid phase microextraction (DI-SPME), a new sampling and sample preparation tool for metabolomics, was used in this study for the first time to investigate clinical samples. The results showed alteration of diverse compounds involved in different biochemical pathways. The most significant contribution in changes induced by surgery and applied pharmacotherapy was noticed in metabolic profile of lysophospholipids, triacylglycerols, mediators of platelet aggregation, and linoleic acid metabolites. Two cases of individual response to treatment were also reported.

1. Introduction

Over recent years '-omics' analyses have been successfully applied in drug discovery and development [1], clinical diagnostics, and toxicology [2]. Metabolomics refers to the analysis of low molecular weight compounds in biological system, including body fluids, tissues, or cells. The diversity of such compounds in addition to proteomics or transcriptomics allows for better understanding of the mechanisms and dynamics responsible for both physiological and pathological reactions. The main objective of metabolic fingerprinting is to screen metabolite changes induced by external stimuli and find a model, or congeries of analytes, which characterize this response [3]. The large variation in chemical and physical properties of compounds as well as the wide range of their concentration (pM–mM) [4] often requires use of different analytical approaches in order to increase metabolite coverage. While highly sensitive instruments provide several opportunities for metabolic studies, they demand appropriate sample preparation. Although metabolomics has rapidly spread

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in the scientific field and new applications have been reported, sample preparation remains one of the most problematic step in the entire process of metabolome analysis. According to a recent review on sample preparation approaches in metabolomics, sample-preparation protocols using conventional techniques such as liquid–liquid extraction (LLE) or solid-phase extraction (SPE) remove proteins and other biological molecules from biological samples and decrease ionization suppression [3,5]. However, in the case of LLE, analysis of polar and non-polar analytes is performed from two different fractions, which increases the number of samples and overall time of analysis. SPE is used for targeted rather than global metabolomics due to its selectivity [5]. To ensure the best coverage of the compounds, protein precipitation (PPT) is a preferable method [5]; however, inefficient sample cleanup can lead to significant ionization suppression [6]. Targeted analysis on the influence of different sample preparation methods on matrix effect showed that suppression affects polar compounds the most, to the extent in which the signal becomes suppressed even below baseline when PPT is used. Moreover, in the case of the latter method, cumulative matrix effect can occur and invalidate the first set of injected samples, including quality control (QC) [6]. To avoid ion suppression, cleaner sample preparation methods should be used.

Solid phase microextraction (SPME) is a sample preparation method based on diffusion of free analyte to extraction phase [7]. For global metabolomics, SPME is mostly used for volatile compounds, while its application for plasma, urine, or blood analysis focuses on pharmacokinetics [8] and ligand–protein studies [9–11]. The use of SPME for targeted analysis mainly arises from the fact that there are a limited number of commercially available coatings suitable for extraction from complex biological samples. Recently, an extensive study on evaluation of SPME coatings for global metabolomics was performed indicating three out of 42 sorbents tested the most suitable for simultaneous extraction of hydrophobic and hydrophylic compounds [12]. The sample preparation step involves only direct extraction from plasma (or other biofluid) followed by desorption in an appropriate solvent. Simplicity of the procedure enables avoiding the loss of analyte as well as their chemical modification during sample preparation. Solvent-free extraction ensures the lack of dilution, which is an important feature considering the low concentrations of most of the endogenous compounds. Since the volume of extraction phase is very small and the nature of SPME extraction is non-exhaustive, the amount of extracted analyte is very small. This feature provides an advantage over exhaustive standard extraction techniques since the amount of co-extracted compounds causing ion suppression is minimal [12]. Biocompatibility of SPME probes used for the aforementioned studies provides restricted access to large biomolecules such as proteins, and prevents fouling of the coating. Due to their biocompatibility and small dimensions, SPME fibers can be used for *in vivo* experiments. In such cases SPME combines several steps of the entire procedure, including sampling, metabolism quenching, and extraction [13]. Due to the restrictions of regulatory institutions regarding human studies, SPME cannot be used for *in vivo* extraction from blood. However, metabolomics studies with the use of collected samples can still be performed. Since the SPME extraction phase equilibrates with a free fraction of analyte, the results obtained provide information about metabolites, which are biologically active and able to interact with receptors.

Excessive bleeding after cardiopulmonary bypass (CPB) is one of the most common complications of cardiac surgery. Tranexamic acid (TXA) is a forefront antifibrinolytic agent used in cardiac surgery [14–16] which competitively inhibits the activation of plasminogen to plasmin and directly inhibits plasmin activity at much higher doses. It was also reported that lysine analogs such as tranexamic acid prevent prothrombotic and proatherogenic lipoprotein(a) (Lp(a)) assembly *in vitro* [17]. On the basis of *in vivo* studies with transgenic mice, it was hypothesized that lysine analogs increase plasma Lp(a) levels by increasing the dissociation of cell-bound apo(a) in combination with reducing Lp(a) catabolism [18]. Recently, several cases of post-operative seizures were reported in patients administered with high dose of TXA who underwent heart surgeries with the use of CPB [19,20]. Moreover, the administration of TXA is associated with clinically significant cerebral vasospasm with acute cerebral hemorrhage [21]. The metabolomic analysis presented herein was performed parallel to pharmacokinetics study on a group of patients undergoing heart surgery with the use of CPB and administered with this antifibrynolytic agent [15]. As mentioned previously, high concentration of TXA may result in various side effects, thus the global view of biochemical changes induced by TXA and surgical procedure could provide new insight into pathways responsible for these reactions.

The objective of this study was to employ SPME for human metabolome analysis in patients undergoing heart 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 benchtop orbitrap mass spectrometer.

2. Materials and methods

2.1. Chemicals and materials

Tranexamic acid (trans-4-(Aminomethyl)-cyclohexanecarboxylic acid) was purchased from Sigma-Aldrich (Oakville, Canada). Acetonitrile (LC/MS grade) and water (LC/MS grade) were purchased from Fisher Scientific (Ottawa, Canada). Prototypes of biocompatible SPME mix-mode probes (C_{18} with benzenesulfonic acid, 45 μm thickness, 1.5 cm length of coating) were provided by Supelco (Bellefonte, PA, USA).

2.2. Patients and blood sampling procedure

Ten patients undergoing cardiac surgery with the use of extracorporeal circulation (cardiopulmonary bypass, CPB) participated in the study. All patients received the standard perioperative care previously developed in Toronto General Hospital. After induction of anesthesia, TXA was administered intravenously by a 30 mg/kg bolus infused over 15 min using an infusion pump followed by an infusion of 16 mg/kg/h until closure of the sternotomy with a 2 mg/kg load within the pump prime. Blood samples were first taken at baseline and then taken 5 min after the bolus. Once the infusion had begun, samples were taken immediately before and after commencing bypass, followed by 30-min intervals whilst on cardiopulmonary bypass, and 5 min after chest closure. Upon discontinuation of the infusion, samples were taken at 1, 2, 3, 4, 6, 8, 10, and 24 h. Each blood sample was collected into standard citrate collecting tubes which were inverted a minimum of five times to ensure proper mixing with anti-coagulant. Samples were randomly assigned a number and thus blindly transported to the

analyzing laboratory. The standard citrate tubes were stored on ice and then centrifuged at 2000*g* for 15 min at 4 °C with the subsequent supernatant frozen and stored at −70 °C until analysis. For metabolomics purpose, two sets of samples were chosen for analysis—before tranexamic acid administration (baseline) and 60 min after being placed onto cardiopulmonary bypass and drug administration. Thus, patients acted as their own controls. These two sets of samples were assigned as non-dosed (ND) and dosed (D) groups.

Exclusion criteria for the studies were documented as allergy to tranexamic acid, renal impairment, creatinine level >130 μM, deep hypothermic circulatory arrest, pre-existing coagulopathy, and pregnancy.

2.3. Sample preparation

Biocompatible mix-mode probes (octadecyl and benzenesulfonic acid groups) were used for the studies. Prior to use, all fibers underwent preconditioning by overnight exposure to methanol: water (1:1, v/v). The SPME experiment was performed using 300 μL plasma aliquots and 60 min extraction time with 1000 rpm vortex agitation (model DVX-2500, VWR International, Mississauga, ON, Canada). Immediately following extraction, fibers were rinsed in purified water for 30 s to remove any remains of biological material from the coating surface. As a desorption solvent, 300 μL acetonitrile:water, (1:1, v/v) was used. Desorption time was 60 min with vortex agitation 1000 rpm. Extracts were further injected to LC–MS system for analysis.

2.4. LC–MS analysis

Analysis of the samples obtained from SPME experiment was performed on the LC–MS system consisting of the Accela autosampler with cooled system tray, Accela LC Pumps, and Exactive Orbitrap mass spectrometer (Thermo, San Jose, CA, USA). A chromatographic reverse phase separation method was performed using pentafluorophenyl column (Supelco Discovery HS F5, 2.1 mm × 100 mm, 3 μm). The injected extract volume was 10 μL. Mobile phase A consisted of water/formic acid (99.9/0.1, v/v) and mobile phase B consisted of acetonitrile/formic acid (99.9/0.1, v/v). The flow rate of mobile phase was 300 μL/min. The following gradient elution was used: 100% A from 0 to 3.0 min, followed by a linear gradient to 10% A from 3.0 to 25.0 min, and an isocratic hold at 10% A until 34.0 min. The total run time was 40 min/sample, including a 6 min column re-equilibration time. MS experiments were performed with an electrospray-ionization orbitrap mass spectrometer operating in positive mode. Detailed information about LC method as well as MS conditions is described elsewhere [12].

QC samples were prepared by pooling 20 μL of aliquots of all samples used in studies. The importance of column equilibration and system conditioning in order to obtain repeatable results has been previously stated [22]; therefore, several injections of QC samples were performed for system equilibration. Extracts obtained after desorption of metabolites were injected in duplicates into LC–MS system in random order. Every eight injections of examined patients' samples were followed by blank and QC injections.

2.5. Multivariate statistics

Multivariate analysis was performed using SIEVE software v1.2.0 (Thermo, San Jose, CA, US). For framing 0.005 mass window, 1.0 min retention time window, maximum number of frames of 20,000, and a minimum signal intensity of 10,000 were used. Initial 1.0 min (column void volume) and final 5.0 min of the chromatographic run time (re-equilibration of the column) were excluded from data processing. Further procedures involved manual peak picking step where each signal reported by the software was verified according to peak shape and abundance in order to remove all variables present in blank samples or those with an unacceptable peak shape. Multivariate analysis containing Principal Component Analysis (PCA) with Pareto scaling and Partial Least Squares Discriminant Analysis (PLS-DA) was performed using SIMCA P+v12.0 software (Umetrics, Sweden). Discriminant compounds with absolute VIP value higher than 2 were selected for identification. The identification was performed through comparison of accurate mass of the feature of interest with data collected in Human Metabolome Database (HMDB) [23]. For searching 0.005 Da window mass was used. Subsequently, polarity (defined by log *P*) of all hits found in HMDB was verified against experimental retention time to reduce the number of potential compounds. In the next step, the experimental extracted ion chromatograms (XIC) were compared with simulated spectra of the respective hits found in the HMDB. Xcalibur software Version 2.1 (Thermo, San Jose, CA, US) was used for this analysis.

3. Results and discussion

3.1. Metabolome profile in dosed (D) vs. non-dosed (ND) patients

Prior to PCA, chromatographic alignment was employed. In all cases alignment score was >0.9 and for most cases the alignment score was >0.98. The software also enabled the finding of metabolites with significantly different intensities in studied groups by determination of *p*-value (*t*-test) for the expression ratio of each variable. The statistical level of confidence was 95%. The number of detected molecular features was 1009.

Footprinting analysis requires very careful control of data quality in order to avoid analysis of changes caused by instrumental errors and to obtain repeatable results. Blank and QC samples arranged throughout the entire sequence show very good clustering, thereby indicating good system stability (Fig. 1). The details of intensity, mass accuracy and retention time stability in the QC samples can be found in the Supplementary information.

Based on the obtained data, three principal components were found using cross-validation. The created PCA model (Fig. 1) presents good separation between samples obtained from the patients before (non-dosed; ND) and during surgery and drug administration (dosed; D) as well as the existence of four samples outside the Hotelling T^2 ellipse symbolizing 95% confidence level. The observed outliers represent samples obtained from two patients (7D and 9D) measured in duplicates. The first three principal components explain ca. 60% of the model variation (PC1 34%, PC2 14%, and PC3 10%).

In order to validate the data and find specific features discriminating observed changes induced by tranexamic acid administration in patients who underwent surgery with the use of cardiopulmonary bypass, PLS-DA analysis was applied (Fig. 2). The supervised analysis confirmed existence of outliers, which were excluded prior to the identification of molecular features (MFs) contributing the most to organism's response to the medical

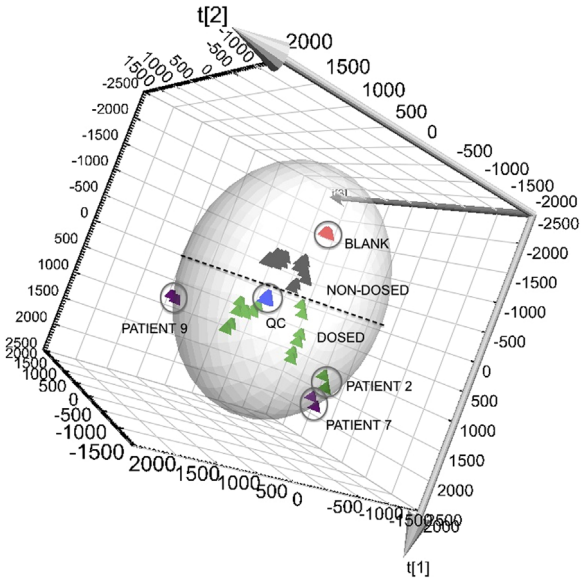

Fig. 1 Principal component analysis for PC1, PC2 and PC3. Good clustering of blank and QC samples and the existence of outliers can be observed.

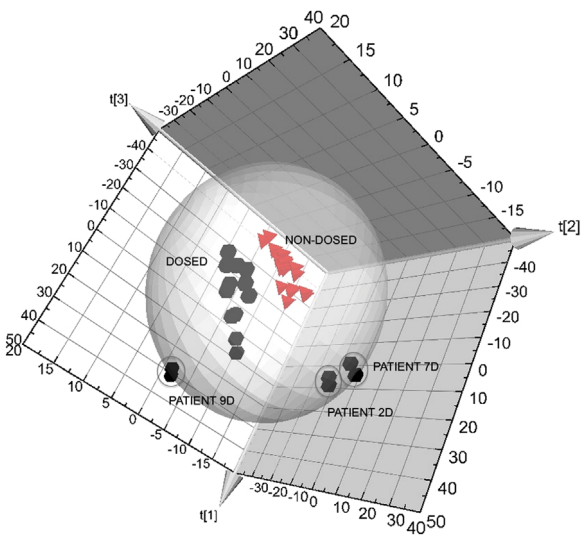

Fig. 2 PLS-DA score plot showing the separation of two cohorts (group of patients before and during surgery and drug administration).

procedures used. However, strong outliers 9D and 7D were further investigated to find individual differences in response to treatment.

Loading plot (Fig. 3) obtained on the basis of PLS-DA plot shows distribution of variables' discriminating response to tranexamic acid treatment and use of CPB. It is evident that several MFs indicate strong contribution in samples' separation. Based on variable influence on projection (VIP) analysis, MFs with the absolute VIP value higher than 2 underwent further identification. According to theory, features with VIP score ≥1 are considered to be statistically significant discriminants for the model under study; however, in the current investigations the threshold was increased to avoid model overfitting due to the small cohort of study participants. Total number of MF with VIP >1 was 115, and with VIP >2 was 38. For the metabolites contributing the most in differentiation between dosed (D) and non-dosed (ND) group, R2 values were close to 1 and Q2 values were above 0.8. This indicates the good abilities of these compounds to explain and predict variances in the model. As expected, the *m/z* for the most contributing compound was found to be tranexamic acid. Additional experiments with the drug standard confirmed retention time and mass accuracy of the tentative variable as TXA.

Among the identified compounds related to the response to tranexamic acid treatment during cardiac surgery in the group of patients studied (Table 1 and appendix to Table 1 in Supplementary information), several classes belong to lipids, with one of the largest being glycerophospholipids. Lysophosphatidylcholines, a subclass of glycerophospholipids, are known to be products of oxidative modification of low-density lipoproteins (LDL) due to hydrolysis of phosphatidylcholine in LDL by group X secretory phospholipase A2 (sPLA2-X) [24]. It was reported that cardiac surgery induces the reduction in circulating LDL [25,26], the increment of Lp(a) level [26,27], and decreased phospholipid concentration in LDL by approximately 38% [25]. At the same time, it was noted that tranexamic acid also influences lipoprotein metabolism by inhibition of Lp(a) binding with LDL [28], platelets [29], and increasing the dissociation of cell bound apo(a) in combination with reducing Lp(a) catabolism [18].

In the studies, statistically significant elevated levels of sphingosine 1-phosphate (S1P) and lysophosphatidic acid (LPL) were observed in the group when patients were dosed with TXA and underwent surgery. Sphingosine 1-phosphate and lysophosphatidic acid are bioactive mediators in platelets aggregation by elevation of $[Ca^{2+}]_i$ concentration and their shape change [30–32]. Additionally, lysophosphatidic acid induces thrombogenic activity in human erythrocytes [33]. It was also reported that S1P stimulates secretion of plasminogen activator-inhibitor type-1 (PAI-1) from adipocytes, which suggests a negative impact on fibrinolysis [34]. Sphingosine 1-phosphate is released at sites of tissue injury [35] and is also involved in the regulation of eicosanoids, important inflammatory mediators production [36] that may explain the increase of S1P level in patients' samples withdrawn during surgery. Two of the main compounds found to contribute to differentiation between dosed and non-dosed groups of patients were mannitol and dopamine sulfate (DOPA). The presence of these compounds is not related to tranexamic acid treatment or response of the organism to cardiopulmonary bypass. Mannitol and dopamine, the most commonly used drugs to reduce the incidence of renal dysfunction [37], were administered to the patients during the surgery. Cardiac surgery with the use of CPB always causes some degree of renal injury and in 2–5% of patients it can have the most severe form—acute renal failure with mortality of 10–20% [38]. The use of CPB also causes a systemic inflammatory response due to exposure of blood to artificial surfaces, ischemia-reperfusion of organs, and release of endotoxin and activation of production of various reactive oxygen species (ROS) [39]. Additionally, mannitol has been found to be a non-specific scavenger of hydroxyl radicals [40]. Another discriminant compound was identified as tryptophan. There was no relationship between the increase of tryptophan concentration and tranexamic acid treatment reported in the literature. However, elevated level of this amino acid can be explained by DOPA administration. Dopamine acts as inhibitor of tryptophan hydroxylase, an enzyme converting tryptophan to 5-hydroxytryptophan (5-HTP), which is an initial metabolite in synthesis of neurotransmitter serotonine [41].

High concentration of tranexamic acid may lead to neurological effects such as seizures due to the competitive inhibition of

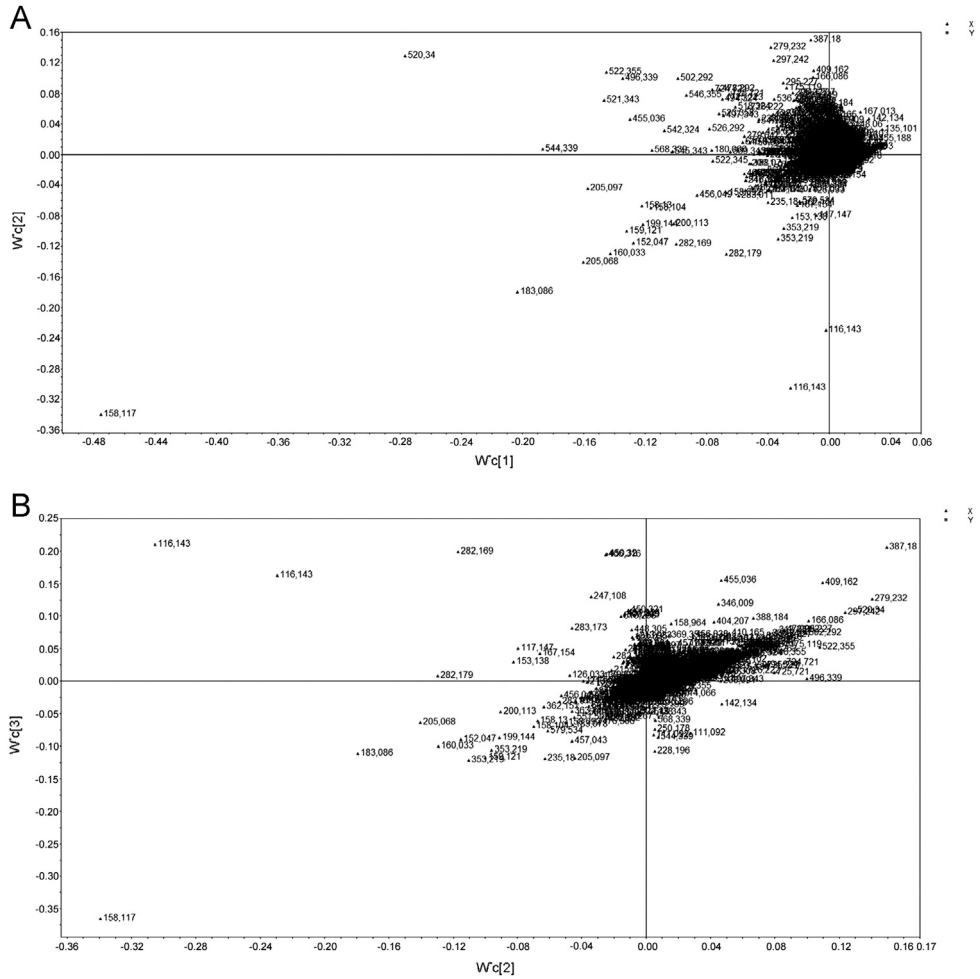

Fig. 3 Loading plots for components 1 and 2 (A) and 2 and 3 (B) showing variables differentiating dosed and non-dosed group of patients.

gamma-aminobutyric acid receptors or to cerebral hemorrhage due to the cerebral vasospasm [21]. Despite the fact that the patients from investigated group showed higher concentration of tranexamic acid than predicted theoretically (> 800 μM) [15], no changes in metabolome profile indicating the existence of neurological disorders were found, which corroborates clinical observations of the patients. The same pharmacokinetic studies initially suggested the possibility of decreased elimination of TXA due to temporary kidney failure, but there was no confirmation in metabolomics analysis. Indeed, the follow up studies performed on the new set of samples with extended sampling time on discontinuation of CPB and TXA bolus reviewed the possible renal dysfunction and showed absolute elimination of TXA several hours after discontinuation of CPB and the drug administration.

3.2. Outliers: individual changes in metabolome profile in patients during surgery

As mentioned previously, two outliers (patients 9 and 7) that belonged to group D were observed in PCA and PLS-DA plots (Figs. 1 and 2, respectively). Analysis of metabolites contributing to the differences between patient 9 and the remainder of patients in group D showed the deviation from the average in the concentration of metabolites previously identified as discriminant compounds: mannitol, lypophospolipids, tryptophan, and eicosapentanoic acid. Herein, positive changes in phospholipid concentration were additionally associated with the increased plasma level of linoleic acid metabolites (9-HODE; 12,13-EpOME; 9,10-Epoxyoctadecenoic acid; 9-HOTE; 9(10)-EpODE; 15(16)-EpODE; 13-HOTE; A-12(13)-EpODE; 9-OxoODE; 13-OxoODE; 17-Hydroxylinolenic acid; 9-HOTE; 9(10)-EpODE; 15(16)-EpODE; 13-HOTE; A-12(13)-EpODE; 9-OxoODE; 9,10-DHOME; 9,10-DiHOME; 13-OxoODE; 9,10-DiHODE; 15,16-DiHODE; 12,13-DiHODE; 9(S)–HPODE; 9,10-DiHODE; 15,16-DiHODE; 12,13-DiHODE; 9(S)-HPODE). Linoleic acid metabolites are also peroxisome proliferator-activated receptor-γ (PPAR-γ) ligands. Some, such as hydroxylinoleic acid (9-HODE), inhibit fibrinolysis by increasing expression of plasminogen activator-inhibitor type-1 (PAI-1) [42].

The 9,10-dihydroxy-12Z-octadecenoic acid (9,10-DHOME; 9,10-DiHOME), metabolite of epoxyoctadecenoic acids (EpOME), exhibited the highest contribution among the abovementioned linoleic acid derivatives in differentiation of outlier 9D. Distribution of the DHOME in the studied cohort is shown in Fig. 4A. There has been no reported evidence that DHOMEs have antifibrinolitic properties; however, DHOMEs are known to be involved in numerous biochemical pathways [43–47]. For example, DHOMEs were found to suppress neutrophil respiratory burst

Table 1 Tentative compounds contributing in differentiation between non-dosed (ND) and dosed (D) groups of patients. List of hits found in the Human Metabolome Database for chosen compounds differentiating studied group of patients. Mass window: 5 ppm.

Class	Subclass	Name
Cycloalkanes	N/A	Tranexamic acid
Amino acids and derivatives	Alpha amino acids and derivatives	L-Tryptophan; N-Decanoylglycine; DOPA sulfate; D-Proline; L-Proline; N-Nonanoylglycine; Tryptophanamide
Alcohols and polyols	Cholines	Undecanoylcholine
Tetrapyrroles and derivatives	Bilirubins	D-Urobilinogen
Indolequinones	N/A	Indole-5,6-quinone
Benzaldehydes	N/A	Gentisate aldehyde
Glycerolipids	Triacylglycerols	*Full list of triacylglycerols can be found in appendix to Table 1 in the Supplementary information
	Monoacylglycerols	D-Glucopyranosiduronic acid MG(24:6(6Z,9Z,12Z,15Z,18Z,21Z)/0:0/0:0) MG(0:0/24:6(6Z,9Z,12Z,15Z,18Z,21Z)/0:0)
	Glycosylglycerols	1,2-Dioctadecanoyl-3-(galactosyl-B-1-6-galactosyl-B-1)-glycerol
Glycerophospholipids	Glycerophosphoethanolamines	*Full list of glycerophosphoethanolamines can be found in appendix to Table 1 in the Supplementary information
	Glycerophosphocholines	*Full list of glycerophosphocholines can be found in appendix to Table 1 in the Supplementary information
	Glycerophosphates	LPA(18:0/0:0); LPA(0:0/18:0); LPA(0:0/18:1(9Z)); LPA(18:1(9Z)/0:0)
Steroids and steroid derivatives	Cholesterols and derivatives	3 Beta,7 alpha-Dihydroxy-5-cholestenoate
	Steroids and steroid derivatives/bile acids, alcohols and derivatives	Chenodeoxyglycocholic acid; Glycoursodeoxycholic acid; 3b,4b,7a,12a-Tetrahydroxy-5b-cholanoic acid; 2b,3a,7a,12a-Tetrahydroxy-5b-cholanoic acid; 3a,6b,7b,12b-Tetrahydroxy-5b-cholanoic acid; 3a,7a,12a,19-Tetrahydroxy-5b-cholanoic acid; 3a,4b,7a,12a-Tetrahydroxy-5b-cholanoic acid; 1b,3a,7b-Trihydroxy-5b-cholanoic acid; 3a,6b,7a,12a-Tetrahydroxy-5b-cholanoic acid; Lithocholic acid glycine conjugate; Glycocholic acid
	Steroidal glycosides	Deoxycholic acid 3-glucuronide
	Gluco/mineralocorticoids, progestogins and derivatives	3a,7b,21-Trihydroxy-5b-cholanoic acid; 3a,6b,7b,12a-Tetrahydroxy-5b-cholanoic acid; 3a,7b,12a-Trihydroxyoxocholanyl-Glycine
Sphingolipids	Neutral glycosphingolipids	Ganglioside GA2 (d18:1/12:0)
	Ceramides	Trihexosylceramide (d18:1/12:0) Ceramide (d18:1/25:0)
	N/A	Sphingosine 1-phosphate
Fatty alcohols	N/A	Mannitol
Fatty acid esters	Acyl carnitines	Docosa-4,7,10,13,16-pentaenoyl carnitine Cervonyl carnitine
Fatty acids and conjugates	Hydroxy fatty acids	9,10-DHOME; 12,13-DHOME
	Branched fatty acids	Alpha-ketoisovaleric acid 2-Methylacetoacetic acid
	Straight chain fatty acids	Glutarate semialdehyde
Prenol lipids	Sesterterpenes	7 alpha-Hydroxy-3-oxo-4-cholestenoate
Keto-acids and derivatives	Short-chain keto acids and derivatives	2-Oxovaleric acid
Sugar acids and derivatives	Sugar amino acids and derivatives	N-Acetyl-7-O-acetylneuraminic acid N-Acetyl-4-O-acetylneuraminic acid N-Acetyl-9-O-acetylneuraminic acid

[43], have proinflammatory effect [44], positive inotropic actions on the isolated rat heart [45], and neutrophil chemotactic activity [46]. In cardiac surgery, due to the contact of patient blood with artificial surfaces of the extracorporeal circuits, activation of the immune system through the neutrophil chemotaxis may occur [47]. In turn, this can lead to postoperative organ dysfunction. Enhanced responsiveness of circulatory neutrophils after cardiac surgery with CPB has been reported previously [47]. Current results may suggest that DHOME can participate in neutrophils activation pathway in patients undergoing heart surgery with CPB

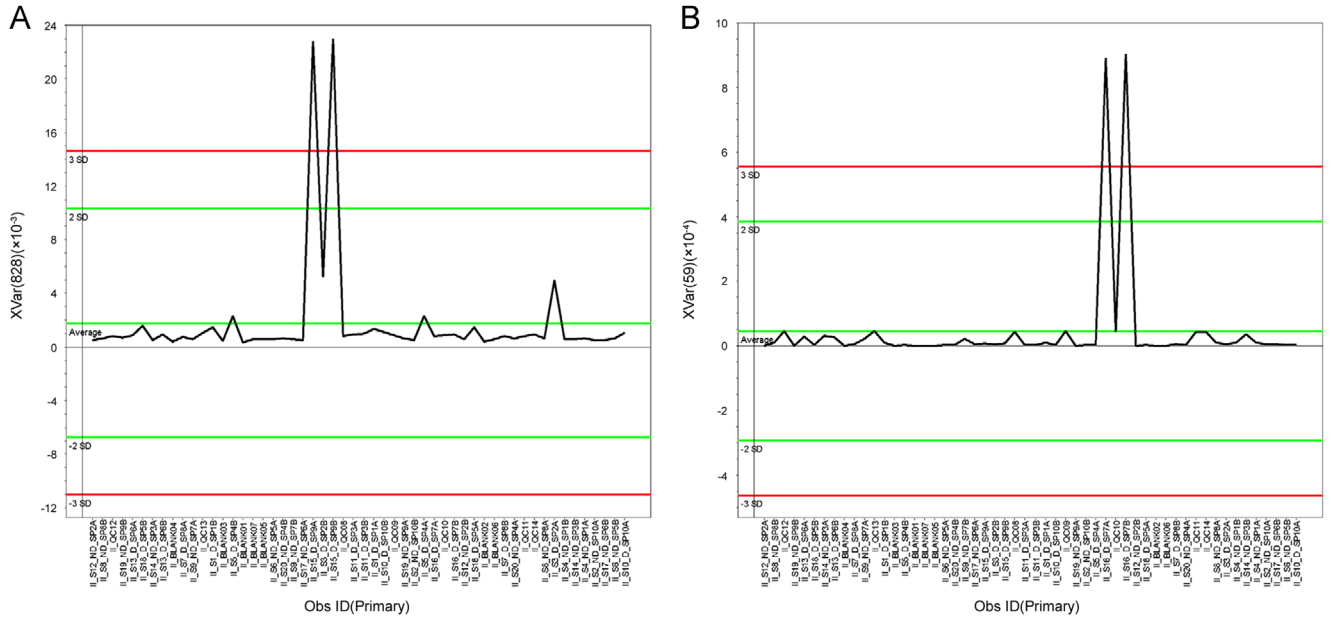

Fig. 4 Individual changes in response to surgery and drug administration. Distribution of *m/z* 315.25 (A) and 450.32 (B) variables among the studied group of patients shows significant increase of their level in plasma samples of patient 9D and patient 7D, respectively.

and concurrent administration of TXA; however, further studies should be performed to verify this theory.

Two pairs of the observed outliers have different projections on PCA and PLS-DA plots (Figs. 1 and 2, respectively). This points to variables characterizing a difference in metabolic profiling of these patients. Indeed, a comparison of metabolites contributing to differentiation between patient 7 and the remainder of group D indicates that the most discriminating compounds belong to bile acids and phosphatidylcholines. As an example, distribution of cholic acid (adduct M+ACN+H; *m/z* 450.3204) in the studied cohort is presented in Fig. 4. Post-operative clinical observation did not show functional liver changes in this patient.

4. Conclusion

Results indicate that SPME coupled with LC–MS platform can be successfully used for studies of human metabolic fingerprinting. With the use of a new extraction phase it is possible to extract compounds with different chemical and physical properties without any prior sample preparation steps directly from plasma aliquots. Since SPME extraction phase is at the equilibrium with a free fraction of analyte, the results obtained provide information about metabolites, which are biologically active and are able to interact with receptors.

The current proof-of-concept study was performed on a small cohort with satisfactory separation between samples collected prior to and during surgery, and administration of tranexamic acid was achieved. The most significant contribution to changes induced by the medical procedure and pharmacotherapy used was observed in metabolic profile of lysophospholipids, mediators of platelet aggregation, triacylglycerols, and linoleic acid metabolites. The analysis of metabolic profiles of patients observed as outliers provides additional insight into individual response to treatment employed or medical condition of the patient, which can be used in personalized therapy. Up-regulation of 9,10-dihydroxy-12Z-octadecenoic acid and/or 12,13-dihydroxy-12Z-octadecenoic acid (9,10-DHOME and 12,13-DHOME, respectively) found in one outlier case possibly suggests that DHOME can play a role in neutrophil activation due to blood contact with artificial circulatory system.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jpha.2013.03.002.

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