

Blood plasma plasmalogens and fatty acids in multiple organ dysfunction syndrome

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Abstract

Introduction: Changes in fatty acid composition as well as in a level of blood plasma plasmalogens in cases of various pathological conditions are evidences of lipid metabolism disorders and can indicate their reasons and degree. The objective of this study was to analyze fatty acids and plasmalogens of blood plasma in patients with multiple organ dysfunction syndrome (MODS).

Methods: Fatty acid ethyl esters and diethyl acetals of fatty aldehydes obtained during sample preparation were analyzed by capillary gas-liquid chromatography.

Results: Marked changes in the plasma fatty acid composition and plasmalogen levels in pa-

tients with MODS were detected.

Conclusions: Based on the detected significant reduction in the plasmalogen levels of blood plasma, a conclusion was made about possible presence of peroxisome dysfunctions in patients with MODS. Peroxisome dysfunction may be one of the reasons of violation of detoxification processes, fatty acids oxidation disorder, prolongation and intensification of the inflammatory process, neurological disorders, and decreased blood antioxidant capacity.

The assumption was made about an important role of fatty acids in disturbance of systemic hemodynamics, assessment of a degree of lipid metabolism disorders and activity of catabolic response.

Key words: Plasmalogens, fatty acids, multiple organ dysfunction syndrome, blood plasma, erythrocyte.

Introduction

Despite a considerable number of publications related to general and specific mechanisms of MODS pathogenesis, methods of correction of metabolic disorders accompanying MODS have not so far sufficiently developed. (1-3) At the same time, in the aspect of lipid metabolism researches, it is important to study the composition of fatty acids (lipid acyl groups) and fatty aldehydes (plasmalogen alkenyl groups with aldehydogenic etheric bonds), which are the structural base of most lipids and involved in metabolic processes, formation of cell membranes, processes of lipid oxygenation and eicosanoid synthesis. (4-6) It should be noted that in many contemporary articles the term fatty alcohol (similarly with vinyl alcohol)

is used to denote an alkenyl group of plasmalogens. (4,7,8) However, aldehydes (with subsequent formation of acetals) are released from plasmalogens during acid hydrolysis that is usually used when analyzing fatty acids. (9,10)

In this study, we analyzed the fatty acid composition and assessed plasmalogen levels of blood plasma as possible participants and markers of nosology of MODS of various etiologies.

Materials and methods

Subjects

Nineteen patients with MODS (mean age 37.6 ± 8.3) of various etiologies treated in the Intensive Care Department of the Mogilev Regional Hospital were studied. The following nosological forms were reasons of MODS development: acute destructive pancreatitis (7 patients); chronic relapsing pancreatitis (1 patient); influenza H1N1, viral, and bacterial bilateral pneumonia (7 persons); severe concomitant injury (1 patient); angiodysplasia of the colon with recurrent intestinal bleeding (1 patient); necrotic shin abscess and septic shock (1 patient), and antiphospholipid syndrome and recurrent pulmonary embolism (1 patient). Blood of 17 apparently healthy volunteers (mean age 38.4 ± 3.3) was used for control purposes.

Besides, there were also investigated abdominal

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aorta fragments and blood plasma of nine dead men (mean age at death 50 ± 6.7). For this purpose, each of the aortas was scraped with a slide plate to get samples of the luminal surface of the vessel (endothelium and subendothelium layer with a thickness about 0.2-0.3 mm) and 3-4 cm³ of anti-coagulated blood were placed in a plastic tube.

Chromatographic analysis

The preanalytical phase of the study comprised separation of erythrocyte component and blood plasma by centrifugation at 5000 rpm for 5 minutes. The separated erythrocytes were twice rinsed in a pH-balanced isotonic solution and repeatedly centrifuged. After that we prepared solutions of derived fatty acids and fatty aldehydes from the fixed volumes of blood plasma and erythrocytes by acid ethanolysis with following hexane extraction. (10) For this purpose we added 0.5 cm³ of concentrated erythrocytes or plasma to 5 cm³ of mixture of ethanol and concentrated (38%) hydrochloric acid (8.75:1.25 by volume).

Lipid ethanolysis reaction was carried out at 60 °C for 1 hour in thermostat. Thereafter the samples were cooled at room temperature for 20 minutes and 1 cm³ of hexane was added to each sample for extraction, which was performed for 10 minutes with gentle stirring. Then the samples were kept for 15 minutes for complete separation of hexane and alcohol layers. Extracts obtained were placed into quartz vials with skrew caps. The samples were stored no longer than one week at -20 °C before analyzing.

Lipids of scrapings of blood vessel luminal surfaces have also been subjected to ethanolysis.

Then various aldehydogenic alkenyl groups (fatty aldehydes) and acyl groups (fatty acids) of lipid molecules were analyzed, which presented in hexane extracts in the form of corresponding diethyl acetals and ethyl esters.

For analyzing the method of gas-liquid chromatography (10) with identification of chemical compounds by flame-ionization detectors was used. Measurements were performed using gas chromatographs (GC-1000 Chromos and Tsvet-800, Russia). To do this, 10 uL chromatographic microsyringe was filled with 5 uL of the hexane extract and it was injected into an evaporator (injector) of the chromatograph.

Analyzed compounds were separated with the aid of a capillary column (60 m, 0.56 mm internal diameter) and SE-30 as a silicone stationary phase (a sorbent film thickness was 0.25 micrometers).

Nitrogen was used as a carrier gas. Chromatographic conditions were the following: tempera-

tures of chromatograph evaporator and detector were 280 °C and 290 °C, respectively; a carrier gas flow rate was 60 cm³/min. Sample injection was performed in split mode with a 1:12 split ratio.

Chromatograms were obtained with the use of a non-linear program of heating of a column thermostat. At the first stage, separation continued for 30 minutes in an isothermal mode at 150 °C. Then the temperature in the column thermostat was raised to 260 °C in several steps (2 and 4 degrees/min).

Final identification was performed with the aid of gas chromatography-mass spectrometry (9) using a gas chromatograph/mass spectrometer (Thermo Scientific DSQ II, USA) equipped with the similar chromatographic capillary column. A routine ionization mode (energy of ionizing electrons) was 70 eV.

Quantitative assessment of contents of single fatty acids and fatty aldehydes was performed by the normalization method (the peak area in the chromatogram corresponding to a certain analyte, was represented as a percentage of the total area of fatty acid and fatty aldehyde peaks) after their conversion into corresponding ethyl esters and diethyl acetals. (8) In such a case, an amount of the analyte was nearly in line with its mass percentage of the total sum of fatty acids and fatty aldehydes.

The total amount of diethyl acetals was expressed as a sum of hexadecyl (C16:0) aldehyde diethyl acetal, octadecyl (C18:0) aldehyde diethyl acetal and octadecenyl (C18:1) aldehyde diethyl acetal.

Simultaneously, total cholesterol content of blood plasma was measured with the aid of a chemistry analyser (Roche Hitachi 912, Japan).

Statistical analysis

Data obtained were presented in the form of the means and corresponding confidence intervals at $p=0.05$ for groups under comparison. Their normality was confirmed by Kolmogorov-Smirnov and Shapiro-Wilk tests ($p=0.05$). (11)

In case of impossibility of confirmation of the normality, data were presented as a median and an interquartile range (Me [LQ; UQ], where Me is the median; LQ is a low quartile; UQ is an upper quartile).

The significance of sample differences were assessed with the Mann-Whitney U test enabling us to operate with moderate-sized samples. (11) The differences were considered to be significant at $p<0.05$.

Ethical considerations

Our researches were performed in accordance with standards of Good Clinical Practice and principles

of the Declaration of Helsinki and rationally planned allowing for minimization of invasive procedures for examined patients since necessary data could not be obtained without involving human participants.

The researches were based on laboratory data and deep knowledge of the problem and promoted obtaining new data about the pathology under study and results to improve diagnosis and treatment. Expected benefits of these researches outweighed potential risks, which were minimal and did not exceed those existing at routine diagnostic procedures related to biochemical blood examinations.

The study was approved by the Ethical Committee of the Mogilev Regional Hospital, Belarus (President Dr. Gennady M. Karpelev), Protocol No. 2 on 15 September 2016.

Results

Marked changes in the fatty acid and fatty aldehyde composition of plasma in patients with MODS were detected (**Table 1**).

The relative content of fatty aldehyde diethyl acetals against the total amount of fatty acid ethyl esters and fatty aldehyde diethyl acetals in the MODS group was just $0.96 \pm 0.33\%$ vs. $2.09 \pm 0.40\%$ ($p < 0.001$) in the control group.

The relative content of polyunsaturated fatty acids (PUFAs) such as linoleic (C18:2), dihomogamma-linolenic (C20:3), arachidonic (C20:4), and docosahexaenoic (C22:6) ones was significantly decreased as well as that of saturated stearic (C18:0) fatty acid. At the same time, the level of monounsaturated fatty acids was increased, so the relative content of oleic (C18:1) and palmitoleic (C16:1) fatty acids in blood plasma was half as much again as that in the control group.

Discussion

In this study a significant reduction in a level of aldehydogenic alkenyl group (fatty aldehydes) has been detected against that of acyl group (fatty acids) in the blood plasma lipid composition of patients with MODS, which indicated a reduction in the level of plasmalogens.

A primary hydroxyl group in glycerol of plasmalogens was substituted by the aldehydogenic alkenyl group in the form of vinyl ether (4,8) rather than by the acyl group as in diacyl phospholipids.

It is determined that diacyl phospholipids and plasmalogens participate in the metabolism of PUFAs with a large number of double bonds such as arachidonic (C20:4) and docosahexaenoic (C22:6) ones and act as an intermediate depot, through which these fatty acids are transported to

the cell membrane. (4,7) Thus, a ratio of levels of fatty aldehydes and of sum of corresponding polyunsaturated fatty acids of blood plasma can reflect changes in a fraction of plasmalogens in comparison with that of diacyl phospholipids.

The ratio of the fatty aldehyde level to the total amount of arachidonic (C20:4) and docosahexaenoic (C22:6) PUFAs of MODS patients was 0.16 ± 0.04 , whereas this ratio was 0.27 ± 0.06 ($p < 0.001$) in the control group. Thus, it can be concluded that the level of plasmalogens against diacyl phospholipids of MODS patients was reduced by almost 40% as compared to the control group (**Figure 1**). In addition the ratio of the fatty aldehyde level to the stearic (C18:0) acid of blood plasma (the most significant of which is contained in the diacyl phospholipids (12)) was 0.09 ± 0.02 in patients with MODS and 0.18 ± 0.03 in the control. That also indicated a significant reduction of plasmalogens in relation to diacyl phospholipids.

Contemporary literature sources (4,8) contain information proving that molecules of plasmalogens can be easily oxidized by reactive oxygen species. It is known (4,8) that oxidation of fatty aldehyde in the sn-1 position of a glycerol molecule residue by reactive oxygen species decreases probability of oxidation of the moiety of polyunsaturated fatty acids located in the sn-2 position.

Thus, the oxidative stress, accompanying systemic inflammatory response, may be one of the reasons for decline of plasmalogen levels.

However, the share of plasmalogens in erythrocytes from patients with MODS was even slightly increased ($16.46 \pm 1.57\%$ in patients with MODS and $13.79 \pm 1.73\%$ in the control, $p < 0.05$), despite the fact (13) that the erythrocytes circulate in the bloodstream for a long time, and its phospholipid exchange with lipoproteins is very limited. The share of plasmalogens in erythrocytes is increased apparently due to the higher activity of the calcium-dependent A2 phospholipase in comparison with plasmalogen-selective phospholipase A2.

On the other hand, plasmalogen deficiency is known to be an important marker of peroxisome dysfunction. Initial stages of synthesis of these lipids have been shown to take place in peroxisomes which functional activity determines its content in tissues. (4,7,8)

At present, the total cholesterol level is indicated as one of the peroxisomal dysfunction markers. The cholesterol level reduction was observed in blood plasma of patients with diseases associated with peroxisome biogenesis disorders. (14) A decrease in the cholesterol content was detected in blood plasma and some tissues of experimental an-

imals with deficiency of these organelles. (15) The connection between peroxisomes and the cholesterol synthesis is also confirmed by the fact that they are especially numerous in the cells involved in cholesterol metabolism. (16)

Our research showed reduced plasma total cholesterol level (124.6 ± 28.7 mg/dL) in MODS patients, which may be one of the evidences of peroxisome dysfunction in view of the above.

Thus, peroxisomal dysfunction may play an important role in development of critical states associated with MODS. Because of the central role of peroxisomes in the catabolism of inflammatory lipid mediators, (17,18) the peroxisomal function impairment can contribute significantly to the prolongation and intensification of the inflammatory process. In addition, peroxisome dysfunction may be one of the reasons of violation of detoxification processes, (16) neurological disorders, (4,8) fatty acid oxidation disorder, (16) and decreased blood antioxidant capacity, (16) which is also related to depression of catalase activity. Furthermore, (4) the decrease of plasmalogen levels in the organism may also explain the violation of lung function and the development of respiratory distress syndrome.

In this case, treatment of these patients may include administration of medications stimulating peroxisome proliferation. So, the existing concept of metabolic correction in the case of MODS can be supplemented with new treatment methods based on more fundamental understanding of the MODS pathogenesis.

As we noted earlier, (19,20) the fatty acid composition of blood plasma in patients with MODS (**Table 1**) shifted to the typical composition of triglycerides of adipose (21) and muscular (22) tissue, where monounsaturated oleic (C18:1) fatty acid predominates, but contents of saturated stearic (C18:0) acid, and especially PUFAs were significantly less represented. Thus, the specific nature of the fatty acid composition in patients with MODS is related to activation of an intracellular lipase system. Fatty acids are released by adipocytes, myocytes, and other cellular elements containing neutral fats into systemic circulation, which results in a significant increase in the monounsaturated acid levels in blood plasma. At the same time, (23) the release of cytokines promotes reduction of utilization of fatty acids and triglycerides through inhibition of lipoprotein lipase activity.

Since the activation of lipolysis in adipose depots is mainly caused by stress (24) and pro-inflammatory cytokines TNF and IL-6 influence, (23,25) levels of monounsaturated fatty acids may to some extent reflect the activity of a stress reac-

tion and degree of catabolic response.

In our opinion, these processes result in an imbalance between monounsaturated and polyunsaturated fatty acids in vascular endothelial cells and subsequent changes in metabolism of eicosanoids and other lipid vasoactive mediators. So, a higher portion of monounsaturated fatty acids (in comparison with other ones) can be one of the reasons of disturbances of systemic hemodynamics in MODS patients. Thus, one can assume that the high relative content of oleic and palmitoleic acids can be considered as a marker of systemic hemodynamic disorders.

It should be noted that changes in fatty acid composition of blood plasma samples in patients with MODS are so significant that they become similar to postmortem plasma samples on this parameter. Relative levels of linoleic (C18:2) and arachidonic (C20:4) PUFAs in blood plasma of people who died due to various reasons as well as those of MODS patients are reduced (up to 17.27 [15.74; 21.50]%, $p < 0.001$ and 4.71 [4.24; 5.75]%, $p < 0.05$, respectively). Furthermore, postmortem plasma samples also have a reduced level of saturated stearic (C18:0) acid (up to 9.72 [8.27; 10.24]%, $p < 0.05$) and considerably increased levels of oleic (C18:1) and palmitoleic (C16:1) monounsaturated fatty acids (up to 23.72 [21.94; 26.86]%, $p < 0.01$ and 3.11 [2.82; 3.82]%, $p < 0.001$, respectively). The degree of fatty acid composition change in postmortem blood samples presumably primarily depend on severity and duration of a previous critical and serious condition.

Besides, the composition of fatty acids in blood plasma in case of MODS is similar to that of a luminal layer of the artery wall, in which were marked high levels of monounsaturated palmitoleic (C16:1) and oleic (C18:1) acids (4.29 [3.58; 4.96]% and 35.11 [32.80; 38.76]%, respectively) and low levels of saturated stearic (C18:0) acid (9.36 [8.39; 10.27]%) and PUFAs (C18:2, C20:4, C22:6, and C20:3) fatty acids are 13.50 [13.05; 14.15]%, 2.85 [2.25; 4.79]%, 1.16 [0.84; 1.55]% and 0.44 [0.41; 0.86]%, respectively). This fact indicates a decrease in the influence of intertissue differences in the lipid composition on metabolic processes.

Thus, blood system disorders emerging in critical conditions require timely metabolic correction aimed, inter alia, at normalization of the lipid metabolism.

Acknowledgement

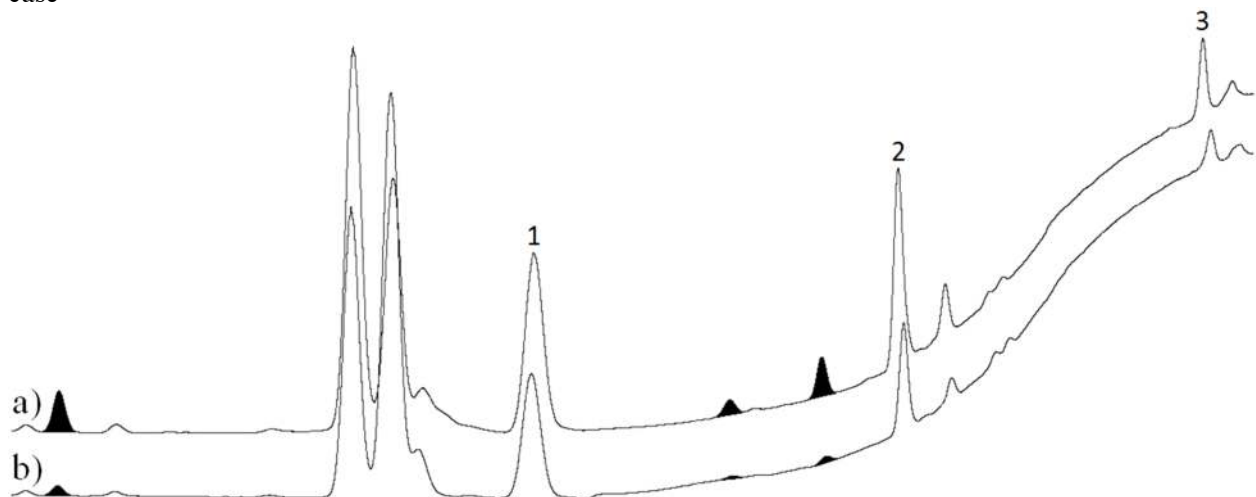
Funding information: self-funding. The authors declare that they have no competing interests.

Table 1. Fatty acid and fatty aldehyde composition of plasma lipids in MODS

Fatty acids and fatty aldehyde	Control, %	MODS, %
Hexadecyl (C16:0) aldehyde	1.19±0.21	0.59±0.20***
Octadecyl (C18:0) aldehyde	0.66±0.14	0.29±0.10***
Octadecenyl (C18:1) aldehyde	0.23±0.07	0.08±0.03**
Myristic (C14:0) acid	0.63±0.11	0.78±0.22
Palmitic (C16:0) acid	26.28±1.51	27.36±1.15
Margaric (C17:0) acid	0.32±0.04	0.31±0.04
Stearic (C18:0) acid	11.73±0.62	10.43±1.02**
Palmitoleic (C16:1) acid	1.52±0.29	2.51±0.39***
Oleic (C18:1) acid	16.19±1.20	24.95±2.17***
Linoleic (C18:2) acid	29.73±2.17	23.75±1.77***
Dihomo- γ -linolenic (C20:3) acid	1.12±0.17	0.82±0.20*
Arachidonic (C20:4) acid	6.05±0.63	4.39±0.75**
Docosahexaenoic (C22:6) acid	2.18±0.42	1.49±0.26**

Legend: The differences were significant * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

Figure 1. Chromatograms illustrating reduction of the fatty aldehyde level in blood plasma in the MODS case



Legend: a)=a healthy volunteer; b)=a MODS patient. Peaks marked in black correspond to diethyl acetals of fatty aldehydes. Fatty acids: 1=C18:0; 2=C20:4; 3=C22:6.

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