Sphingomyelins and ceramides of umbilical cord vein and their alterations in preeclampsia

LECH ROMANOWICZ, ZOFIA GALEWSKA, STEFAN JAWORSKI

Abstract
Preeclampsia is accompanied by an extensive remodeling of the extracellular matrix of the umbilical cord. First of all it is accompanied by an increase of collagen content in the umbilical cord artery and in Wharton’s jelly. Furthermore preeclampsia distinctly reduces proteolytic and gelatinolytic activity, especially after activation with various agents. It was decided to determine sphingomyelins and ceramides content and their polymorphism in umbilical cord vein. Studies were performed on the umbilical cord vein taken from 10 newborns delivered by healthy mothers and 10 newborns delivered by mothers with preeclampsia. Sphingomyelins and ceramides were isolated by Thin Layer Chromatography, fatty acids were liberated by basic hydrolysis and analyzed by HPLC of their p-bromophenacyl derivatives using detection on 254 nm. It was found that preeclampsia decreased in sphingomyelins and ceramides content in umbilical cord vein. Saturated fatty acids were the main group of fatty acids incorporated to sphingomyelins and ceramides from both control and preeclamptic tissues. The estimation of sphingomyelins to ceramides relationship allowed to find that preeclampsia caused significant increase in sphingomyelins relative content in umbilical vein wall.

Key words: sphingomyelins, ceramides, preeclampsia, umbilical cord vein

Introduction
Lipids constitute a large group of arterial wall compounds. They are main components of plasma and intracellular membranes which divides cell inside into many compartments. The lipid composition of arterial walls change during development and ageing and pathological processes. Especially atherosclerosis is accompanied by lipid accumulation in arterial wall [1].

Lipids constitute about 50% of the mass of most animal cell membranes, nearly all of the remainder being protein. The most abundant membrane lipids are the phospholipids with polar head group and two hydrophobic tails – both saturated and unsaturated fatty acids of different length. Four major phospholipids predominate in the plasma membrane of many mammalian cells: phosphatidylcholine, phosphatidylethanolamine, phosphatidylycerine, and sphingomyeline. Together these four phospholipids constitute more than half the mass of lipid in most membranes. Other phospholipids, such as the inositol phospholipids, are present in smaller quantities but are functionally very important. They have crucial role in cell signaling [2].

Normal human arterial wall contains significant amounts of lipids. For instance aorta of adult subject contains about 7.15 mg of lipids per gram of fresh tissue. Most of them are phospholipids (4.28 mg). The other are: cholesterol esters (1.27 mg), cholesterol (1.0 mg) and triacylglycerols (0.59 mg) [3]. There is some data on phospholipids of umbilical cord vessels [4] but there is not much information about sphingomyelins and ceramides. So we decided to isolate, fractionate and determine those class of lipids from umbilical cord vein taken from newborns of healthy mothers and those with preeclampsia.

Materials and methods
The study protocol was approved by the Bioethical Committee of the Medical University of Białystok.

Tissue material. Studies were performed on the umbilical cord vein (UCV) taken from 20 newborns. In all the cases 20 cm long sections of UCV were excised beginning from their placental end, and carefully separated from the surrounding Wharton’s jelly.

The control material was taken from 10 newborns delivered by healthy mothers aged 23-36 with normal blood pressure (systolic 100-135 mm Hg, diastolic 60-80 mm Hg). The mothers presented no symptoms of edema or renal failure. The mean body weight of the newborns was 3560 ± 396 g.

The 10 investigated newborns were delivered by mothers aged 19-31 with preeclampsia, diagnosed according to the criteria accepted by the Organisation Gestosis [5]. All patients demonstrated an elevation of blood pressure (systolic greater than 140 mm Hg, diastolic greater than 90 mm Hg) and proteinuria (greater than trace). All cases of patients with cardio-vascular, renal and metabolic diseases were excluded. The mean body weight of these newborns was equal to 3 205 ± 597 g.

Lipids were isolated according to method described by van der Vusse and colleagues [6, 7]. Sphingomyelins [8] and ceramides [9, 10] were separated by thin layer chromatography. Next they were eluted from gel and were hydrolyzed in 2M KOH according to Engelmann et al [11]. Para-bromophenacyl esters of free fatty acids after hydrolysis were prepared as described by Aoyama and Sato [12]. Separation and determination was performed by reversed-phase high-performance liquid chromatography [11] with methanol: TEAP buffer: H2O: acetonitril mixture in gradient mode on RP-18 column at 40°C with detection at 254 nm [13].

Statistical analysis. Mean values from 10 assays ± standard deviations (SD) were calculated. Particular fatty acid content in sphingomyelins and ceramides from umbilical cord artery wall was expressed in mol%. The results were submitted to statistical analysis with the use of Student’s “t” test, accepting p < 0.05 as significant.

Department of Medical Biochemistry and Department of Gynecology, Medical University of Białystok
Results

Thin layer chromatography of isolated lipids from umbilical cord vein (UCV) allowed separating sphingomyelins or products of their cleavage – ceramides from other neutral and polar lipids. After elution from gel, basic hydrolysis of those esters for liberation of potassium salts of fatty acids and derivatization they were separated and determined by HPLC. Used method allowed to isolate Saturated Fatty Acids (SAFA): lauric, myristic, palmitic, stearic and arachidic acid, MonoUnsaturated Fatty Acids (MUFA): myristoleic, palmitoleic and oleic acid, and PolyUnsaturated Fatty Acids (PUFA): linolic, linoleic, arachidonic, eicosapentaenoic and docosahexaenoic acid. We determined content and molecular polymorphism of sphingomyelins and ceramides.

Sphingomyelins

Figure 1 shows that UCV is characterized by more than two-fold decrease in sphingomyelin content in comparison to this vessel wall taken from newborns delivered by healthy mothers.

Table 1A. Fatty acid content in sphingomyelins of umbilical cord vein in nanomole per gram of tissue (* p < 0.001)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control</th>
<th>Preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 12:0</td>
<td>273.73 ± 11.32</td>
<td>48.30 ± 9.14 *</td>
</tr>
<tr>
<td>C 14:0</td>
<td>158.54 ± 8.58</td>
<td>25.70 ± 6.09 *</td>
</tr>
<tr>
<td>C 16:0</td>
<td>127.83 ± 9.81</td>
<td>41.18 ± 9.02 *</td>
</tr>
<tr>
<td>C 18:0</td>
<td>3844.14 ± 362.19</td>
<td>1672.24 ± 334.57 *</td>
</tr>
<tr>
<td>C 20:0</td>
<td>849.32 ± 172.37</td>
<td>1324.34 ± 188.04 *</td>
</tr>
</tbody>
</table>

SAFA
MUFA
PUFA

The highest amount of SAFA in both kinds of material we observed. The lowest amount of MUFA in control UCV was detected. But preeclampsia evoked the deepest decrease in the content of PUFA in sphingomyelins isolated from UCV wall. Almost five-fold decrease was found and PUFA became the group of fatty acids with the lowest content in sphingomyelins extracted from UCV wall.

Comparison of fatty acid percentage relationship in sphingomyelins isolated from UCV, presented in Table 2A, divers depending on kind of fatty acid. First of all stearic acid is the most abundant fatty acid in sphingomyelins, independently on kind of material. It was more than 40% of all determined fatty acids. The next was myristoleic acid and linoleic acid in control UCV and arachidic acid in preeclamptic material. Other fatty acids formed only low part of percentage, less than 5%. It is of interest that preeclampsia divers the direction of changes these relationships. Only stearic acid from SAFA, myristoleic acid from MUFA and linoleic acid from PUFA percentage does not change. Preeclampsia enhanced percentage contribution of arachidic acid from SAFA, palmitoleic acid and oleic acid from MUFA and only eicosapentaenoic acid from PUFA.
Table 2A. Fatty acid composition of sphingomyelins from umbilical cord vein in mole% (* p < 0.001)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control</th>
<th>Preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 12:0</td>
<td>2.90 ± 0.24</td>
<td>1.05 ± 0.22 *</td>
</tr>
<tr>
<td>C 14:0</td>
<td>1.68 ± 0.13</td>
<td>0.56 ± 0.12 *</td>
</tr>
<tr>
<td>C 16:0</td>
<td>1.35 ± 0.25</td>
<td>0.90 ± 0.21 *</td>
</tr>
<tr>
<td>C 18:0</td>
<td>40.74 ± 7.56</td>
<td>36.50 ± 7.28</td>
</tr>
<tr>
<td>C 20:0</td>
<td>9.00 ± 1.97</td>
<td>28.91 ± 2.39 *</td>
</tr>
</tbody>
</table>

Table 2B. Relationship of saturated (SAFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids from sphingomyelins of umbilical cord vein in mole% (* p < 0.001)

<table>
<thead>
<tr>
<th>Fatty acid type</th>
<th>Control</th>
<th>Preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAFA</td>
<td>55.8 ± 13.2</td>
<td>67.9 ± 14.3</td>
</tr>
<tr>
<td>MUFA</td>
<td>20.1 ± 4.3</td>
<td>22.0 ± 4.4</td>
</tr>
<tr>
<td>PUFA</td>
<td>24.2 ± 2.1</td>
<td>10.1 ± 2.0 *</td>
</tr>
</tbody>
</table>

Table 3A. Fatty acid content in ceramides of umbilical cord vein in nanomole per gram of tissue (* p < 0.001)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control</th>
<th>Preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>C 12:0</td>
<td>845.4 ± 17.2</td>
<td>76.3 ± 16.3 *</td>
</tr>
<tr>
<td>C 14:0</td>
<td>108.8 ± 6.7</td>
<td>22.3 ± 5.2 *</td>
</tr>
<tr>
<td>C 16:0</td>
<td>397.7 ± 31.0</td>
<td>132.2 ± 27.3 *</td>
</tr>
<tr>
<td>C 18:0</td>
<td>3000.0 ± 235.8</td>
<td>1083.3 ± 221.4 *</td>
</tr>
<tr>
<td>C 20:0</td>
<td>789.7 ± 94.7</td>
<td>423.9 ± 91.8 *</td>
</tr>
</tbody>
</table>

Table 3B. The amount of saturated (SAFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids from ceramides of umbilical cord vein in nanomole per gram of tissue (* p < 0.001)

<table>
<thead>
<tr>
<th>Fatty acid type</th>
<th>Control</th>
<th>Preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAFA</td>
<td>5142 ± 408</td>
<td>1738 ± 372 *</td>
</tr>
<tr>
<td>MUFA</td>
<td>799 ± 97</td>
<td>428 ± 91 *</td>
</tr>
<tr>
<td>PUFA</td>
<td>2063 ± 60</td>
<td>217 ± 51 *</td>
</tr>
</tbody>
</table>

Ceramides

Figure 2 shows that preeclampsia decreased ceramide content in UCV wall about three times. Its amount was almost twice lower than sphingomyelins in preeclamptic subjects.

All other fatty acid percentage contribution in sphingomyelins extracted from UCV wall was lowered by this pregnancy complication.

Table 2B indicates that saturated fatty acids are more than 50% of all fatty acids contained in sphingomyelins extracted from both control and preeclamptic UCV wall. Unsaturated fatty acids – MUFA and PUFA – formed that class of lipids in almost equal percentage in control material. The significant decrease in PUFA percentage (more than two-fold) in sphingomyelins isolated from preeclamptic UCA wall was found only.

Preeclampsia increased sphingomyelin to ceramide content ratio to 1.92 from 1.18 in UCV wall taken from newborn delivered by healthy mothers.

As can be seen from Table 3A, ceramides extracted from UCV wall contained all determined fatty acids. Stearic acid predominated among not only in SAFA but in all detected fatty acids in that fraction of lipids isolated from control tissue. More than one-third lower portion of linoleic acid, lauric acid and arachidic acid was found.
ramides, because six of them percentage increased with simul-

taneous ratio of fatty acids contained in preeclamptic UCV wall ce-
general preeclampsia evoked opposite changes in proportio-
ceramides and arachidic acid percentage to more than 17%. In

stearic acid percentage enlarged to 45% in preeclamptic UCV
lauric acid and arachidic acid we found. It is of interest that
fatty acids for control subjects. About 10% of linoleic acid,
in sphingomyelins stearic acid was present in the highest
fatty acid contained in ceramides isolated from UCV wall. As

content in ceramides isolated from preeclamptic vessel wall
and preeclamptic UCV wall. The highest reduction of PUFA
content of MUFA and the lowest one of PUFA in both control

myelins also for ceramides SAFA formed group of fatty acids
all fatty acid content but still stearic acid predominated.

Other fatty acids were represented in even lower amount in
control UCV. Preeclampsia evoked huge decrease in almost
all fatty acid content but still stearic acid predominated. Arachidic acid, oleic acid, myristoleic acid and palmitic acid
was detected in lower amount. Other fatty acids were pre-

Table 4A. Fatty acid composition of ceramides from umbilical cord vein in mole% (* p < 0.001)

<table>
<thead>
<tr>
<th>Fatty acid</th>
<th>Control</th>
<th>Preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C 12:0</td>
<td>10.57 ± 0.93</td>
<td>3.20 ± 0.74 *</td>
</tr>
<tr>
<td>C 14:0</td>
<td>1.36 ± 0.26</td>
<td>0.94 ± 0.22 *</td>
</tr>
<tr>
<td>C 16:0</td>
<td>4.97 ± 1.04</td>
<td>5.54 ± 1.10</td>
</tr>
<tr>
<td>C 18:0</td>
<td>37.52 ± 8.22</td>
<td>45.46 ± 9.09</td>
</tr>
<tr>
<td>C 20:0</td>
<td>9.88 ± 2.09</td>
<td>17.79 ± 2.34 *</td>
</tr>
<tr>
<td>MUFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C 14:1</td>
<td>4.00 ± 0.92</td>
<td>7.01 ± 1.03 *</td>
</tr>
<tr>
<td>C 16:1</td>
<td>1.71 ± 0.36</td>
<td>1.84 ± 0.38</td>
</tr>
<tr>
<td>C 18:1</td>
<td>4.19 ± 0.96</td>
<td>9.12 ± 1.07 *</td>
</tr>
<tr>
<td>PUFA</td>
<td></td>
<td></td>
</tr>
<tr>
<td>C 18:2</td>
<td>11.57 ± 0.51</td>
<td>1.50 ± 0.42 *</td>
</tr>
<tr>
<td>C 18:3</td>
<td>5.33 ± 0.49</td>
<td>2.03 ± 0.42 *</td>
</tr>
<tr>
<td>C 20:4</td>
<td>0.52 ± 0.12</td>
<td>2.36 ± 0.18</td>
</tr>
<tr>
<td>C 20:5</td>
<td>8.02 ± 0.53</td>
<td>2.03 ± 0.47 *</td>
</tr>
<tr>
<td>C 22:6</td>
<td>0.35 ± 0.14</td>
<td>1.18 ± 0.17 *</td>
</tr>
</tbody>
</table>

Other fatty acids were represented in even lower amount in
control UCV. Preeclampsia evoked huge decrease in almost
all fatty acid content but still stearic acid predominated. Arachidic acid, oleic acid, myristoleic acid and palmitic acid
was detected in lower amount. Other fatty acids were pre-

Table 4B. Relationship of saturated (SAFA), monounsaturated (MUFA) and polyunsaturated (PUFA) fatty acids from ceramides of umbilical cord vein in mole% (* p < 0.001)

<table>
<thead>
<tr>
<th>Fatty acid type</th>
<th>Control</th>
<th>Preeclampsia</th>
</tr>
</thead>
<tbody>
<tr>
<td>SAFA</td>
<td>64.3 ± 14.7</td>
<td>72.9 ± 15.2</td>
</tr>
<tr>
<td>MUFA</td>
<td>9.9 ± 2.1</td>
<td>18.0 ± 2.5 *</td>
</tr>
<tr>
<td>PUFA</td>
<td>25.8 ± 2.6</td>
<td>9.1 ± 2.2 *</td>
</tr>
</tbody>
</table>

taneous, five fatty acids percentage decreased and percentage of two of them did not change (Table 4A).

The percentage of SAFA, MUFA and PUFA content of UCV ceramides is present in Table 4B. In both cases (control
and preeclamptic subjects) SAFA were present in the highest
amount in comparison to MUFA and PUFA. Preeclampsia

evoked a slight but not significant increase in SAFA percent-
ge in UCV. We observed opposite changes in MUFA and
PUFA ratio. MUFA percentage increased twice and PUFA per-
centage decreased more than two and a half-fold in ceramides
isolated from preeclamptic UCV wall (Table 4B).

**Discussion**

The vascular system of mother and placenta plays an im-
portant role in the intrauterine development of the fetus. The
umbilical cord forms the connection between the placenta and
the fetus. The cross section of the umbilical cord shows a
specific gross morphology of one vein and two arteries surrounded
by a distinct connective tissue region called Wharton’s jelly. Both arteries lead venous blood from the fetus to the placenta. Umbilical cord vein pipes off fetal blood from the placenta to
the fetus. It is well known that major exchange of all substances between fetus and mother occurs by fetal blood in
placenta. So umbilical cord vein walls have continuous contact
with all substances cruising in fetal blood.

Preeclampsia [14] is the most common, pregnancy-asso-
ciated pathological syndrome. Genetic, immunologic and die-
tary factors may be involved in pathogenesis of preeclampsia.
It is accompanied by significant morphological and functional
alterations in the vessel walls of the uterus and placenta [15].

It is difficult to determine venous wall composition of
healthy newborns. Umbilical cord is the best source of tissue
material for investigation, independently from healthy subject
or with any complication. It was found in our previous studies
that preeclampsia is accompanied by an extensive remodeling
of extracellular matrix of the umbilical cord. The umbilical cord
arteries of newborns delivered by mothers with preeclampsia
contain more than twice the amount of collagen and markedly
less elastin in comparison to corresponding arteries of new-
borns delivered by healthy mothers [16, 17]. The preeclamptic
UCV contains less collagen and more elastin [18]. The changes
in collagen composition are accompanied by an early reduction
of hyaluronic acid in the umbilical cord arterial wall [19], as
well as in Wharton’s jelly [20] and its replacement by sulphated
glycosaminoglycans [19, 20]. It seems that the remodeling of
the umbilical cord vessels may be responsible for the decrease
of blood flow in the fetus of woman with preeclampsia.

Sphingomyelins are very important class of phospholipids.
They build biological membranes especially cell bilayer. They
are also involved in intracellular signal transductions by
hydrolysis with liberation of ceramides. The latter acts as se-
cond messengers. Ceramides can be degraded to free fatty
acids and sphingosine, which can be used to formation of
sphingosine-1-phosphate or to combine with simple sugar (glu-
cose or galactose). Comparing present results with our earlier
studies [21] one can suppose that preeclampsia lowered sphin-
Sphingomyelins and ceramides of umbilical cord vein

gomelin content in cells or restricted its synthesis or lowered cell number per gram of tissue. We observed significant
decrease in sphingomyelin content in preeclamptic UCV wall with simultaneous reduction of its degradation products am-
ount (ceramides and sphingosine). But we also found signifi-
cantly higher content of sphinganine in UCV wall taken from
newborns delivered by mothers with preeclampsia [21]. Sphin-
ganine is one of middle metabolites during sphingomyelin syn-
thesis. Described above extracellular matrix remodeling with
huge changes in content and polymorphism of investigated phospholipids, which are integrated elements of cells, may
indicate deep reorganization of preeclamptic umbilical cord
vessel walls.

It is commonly known that preeclampsia is a syndrome which
results in a reduction in birth weight of newborns. In fetuses with retarded growth Doppler ultrasound has shown evidence of increased peripheral resistance to blood flow in the descending aorta [22]. According to Gennser et al. [23] some cases of intrauterine growth retardation are associated
with maternal hypertension.

It is apparent from the reports of several authors that low
birth weight for the period of gestation may be a predictor of
raised blood pressure in childhood and adult life [23-26]. Barker et al [24] investigated a relationship between the birth
weight and blood pressure in childhood and adult life. In
groups of 9921 10-year old children and 3259 adults in Britain,
systolic blood pressure was inversely related to birth weight.
The association was independent of the period of gestation. It
may be supposed that preeclampsia is a factor which evokes
the initiation of hypertension in utero and its amplification
throughout childhood and adult life.

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versity of Białystok grant No. 3-15601 L.

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contents of both n3 and n6 long-chain polyunsaturated fatty


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