Altered Sphingolipid Composition in Wharton's Jelly of Pre-Eclamptic Newborns

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Key Words

Human umbilical cord · Wharton's jelly · Sphingolipids · Sphingomyelin · Ceramide · Sphingoid bases · Pre-eclampsia

Abstract

Objectives: Wharton's jelly is a myxomatous substance surrounding the umbilical cord (UC) vessels to protect them against extension, bending, twisting and compression. Pre-eclampsia (hypertension, oedema, proteinuria) is the most common pregnancy-associated pathological syndrome. It is accompanied by significant alterations in UC composition. Here we describe the sphingolipids of Wharton's jelly from 10 newborns delivered by healthy mothers and from 10 babies of pre-eclamptic mothers. **Methods:** Thin-layer chromatography, solid-phase extraction and high-performance liquid chromatography were employed. **Results:** Control tissues were abundant in sphingomyelins (Sms) and ceramides (Cers), whereas the amounts of sphingoid bases were distinctly lower. Pre-eclampsia is associated with a significant increase in Sms, Cers, sphingosine, sphinganine, 4-OH-sphinganine, sphingosine 1-phosphate and glycosylated sphingosine. Furthermore, a decrease in sphinganine 1-phosphate was found. Stearate ($C_{18:0}$) is the dominating fatty acid in Sms and Cers of control tissue. In contrast, pre-eclamptic material contained the highest amount of laurate

($C_{12:0}$) in Sms and myristoleate ($C_{14:1}$) in Cers. **Conclusions:** Sphingolipids and some sphingoid bases are bioactive molecules which contribute to the regulation of signal transduction pathways, protein sorting and mediation of cell-to-cell interactions and recognition. The alteration in sphingolipid content may modify the metabolism of Wharton's jelly, resulting in remodelling of its composition.

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Introduction

The vascular system of the umbilical cord (UC) and placenta plays an important role in the intra-uterine development of the fetus. The UC forms a connection between the placenta and the fetus. It is composed of 3 blood vessels of different structure and function; one UC vein (UCV), which transports oxygenated and nutritionally rich blood from the placenta to the fetus and two UC arteries (UCAs), which transport deoxygenated blood and metabolic waste products from the fetus to the placenta. These 3 vessels are surrounded by gelatinous connective tissue, which is referred to as 'Wharton's jelly'. It is composed of a very low number of cells and high amounts of extracellular matrix (ECM) components – mainly collagen, hyaluronate and several sulphated proteoglycans [1, 2].

The large amount of hyaluronate makes this tissue highly hydrated, whereas the abundant content of collagen makes it resistant to extension, bending, twisting and compression evoked by fetal movements and uterine contractions. Furthermore, the ECM of Wharton's jelly is an abundant reservoir of numerous peptide growth factors (PGFs) [3].

Rapid and localized changes in the activity of these factors can be induced by their release from matrix storage and/or by activation of latent forms. The PGFs, in turn, control cell proliferation, differentiation as well as synthesis and remodelling of the ECM. This suggests that the ECM plays a major role in the control of PGF signalling [4].

Since Wharton's jelly contains a low number of cells and very high amounts of ECM components, it may be concluded that the scanty cells are strongly stimulated to produce large amounts of collagen [1], hyaluronate [5] and sulphated proteoglycans [6]. It is well known that the biosynthesis of ECM components is enhanced by several PGFs – mainly insulin-like growth factor [7], fibroblast growth factor [8] and transforming growth factor- β [9]. The PGFs may accumulate within Wharton's jelly to promote the synthesis of large amounts of ECM. Furthermore, Mitchell et al. [10] found that stromal cells of Wharton's jelly have properties of potentially multipotent stem cells. Treatment with basic fibroblast growth factor induces these cells to express a neural phenotype [10].

Some products of lipid metabolism may be engaged in this process. The fatty acids (FAs) serve as a source of energy for a number of cells [11]; many are substrates for the synthesis of regulatory molecules: prostaglandins, thromboxanes and leukotrienes [12]. Several lipids (including sphingolipids) are constituents of biological membranes, which separate the cells from the surrounding hydrophilic environment and contribute to organelle structures [13]. Some are substrates for the production of second messengers (e.g. diacylglycerol, inositol 1,4,5-trisphosphate, ceramides), which participate in intracellular signal transduction induced by hormones and PGFs bound to membrane receptors [14]. Thus, alterations in lipid compounds of Wharton's jelly may participate in the deregulation of various cell functions, including overproduction of sulphated glycosaminoglycans [5] or the downregulation of enzymes which participate in their degradation [15].

Pre-eclampsia, the most common pregnancy-associated pathological syndrome [16], is accompanied by significant morphological and functional alterations in the arterial walls of the uterus and the placenta [17]. Our pre-

vious studies showed that pre-eclampsia is accompanied by extensive remodelling of the ECM of the UC [18–22]. The UCAs of newborns delivered by pre-eclamptic mothers contain more than twice the amount of collagen [19] and markedly less elastin [20] compared with corresponding arteries of newborns delivered by healthy mothers. It is accompanied by an early reduction of hyaluronic acid in the UCA wall and its replacement by sulphated glycosaminoglycans [21]. Increases in proportional amounts of sulphated glycosaminoglycans with simultaneous reduction in hyaluronate content were also observed in Wharton's jelly [1]. Such a phenomenon corresponds to an 'early ageing' of the UC tissues. In contrast, only minor changes were found in UCV ECM of pre-eclamptic newborns [22].

Lipids constitute a large group of cell components and their composition changes during development [23], ageing [24] and pathological processes [25]. They constitute about 50% of the mass of most animal cell membranes, nearly all of the remainder being protein [13]. Wharton's jelly is a highly hydrophilic tissue [26]. The main structures which may contain amphipathic lipids are cell membranes. Sphingomyelins (Sms) belong to the most abundant membrane lipids; they contain a polar head (phosphate and -OH groups) and hydrophobic tails, composed of sphingosine hydrocarbon chain and FAs of different length [13, 27].

Sphingolipids are a major class of lipids which are ubiquitous constituents of membranes in eukaryotes. For a long time, they were considered to play primarily structural roles in membrane formation. Presently, it is known that some of them, including ceramides, sphingosine, sphingosine 1-phosphate and ceramide 1-phosphate are bioactive molecules playing roles in cell growth, differentiation, senescence and apoptosis, regulation of signal transduction pathways, protein sorting and intercellular interactions [for reviews, see ref. 28, 29]. Various stimuli such as tumour necrosis factor- α , Fas ligand, interleukin-1, ionizing radiation, phorbol esters, heat stress, oxidative stress and chemotherapeutics induce the formation of ceramides (Cers) [for reviews, see 30, 31].

The sphingolipid composition of Wharton's jelly has not been evaluated till now and its role in pathobiology remains unclear. It was decided to measure the sphingolipid components – Sms, Cers as well as sphingoid bases in their free, phosphorylated and glycosylated forms – in Wharton's jelly of both healthy newborns and those delivered by pre-eclamptic mothers. A possible regulatory role of Cers, sphingosine and sphingosine 1-phosphate (as secondary messengers) in remodelling of ECM is discussed.

Fig. 1. Representative chromatogram of FAs released from an Sm; 1: lauric acid; 2: myristoleic acid; 3: eicosapentaenoic acid; 4: linolenic acid; 5: docosahexaenoic acid; 6: myristic acid; 7: palmitoleic acid; 8: arachidonic acid; 9: linoleic acid; 10: palmitic acid; 11: oleic acid; 12: stearic acid; 13: arachidic acid; 14: nervonic acid, and 15: behenic acid.

Materials and Methods

The study protocol was approved by the Bioethical Committee of the Medical University of Białystok. In each case, informed consent was obtained from the investigated patients.

Tissue Material

Studies were performed on the UCs taken from 20 newborns. The babies were born between 36 and 41 weeks of gestation. In all the cases, 20-cm-long sections of each UC were excised beginning from their placental end, and Wharton's jelly was carefully separated from the UC vessels.

The control material was taken from 10 newborns delivered by healthy mothers aged 20–30 with normal blood pressure (systolic 110–135 mm Hg, diastolic 60–86 mm Hg). The mothers presented no symptoms of edema or renal failure. The mean body weight of the newborns was $3,540 \pm 453$ g.

The pre-eclamptic material was taken from 10 newborns delivered by pre-eclamptic mothers aged 21–38, diagnosed according to the criteria accepted by the Organisation Gestosis [32]. 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 patients with cardiovascular, renal and metabolic diseases were excluded. The mean body weight of these newborns was equal to $3,245 \pm 606$ g. The mean percentage of fetal growth retardation was equal to 8.3%.

Lipid Extraction

Lipids were extracted from the investigated tissues by the method of Folch et al. [33] with modifications described by Van der Vusse et al. [34].

Sphingomyelins. The lipid-containing extracts were submitted to thin-layer chromatography on commercially available plates covered with silica gel (Merck), activated by heating at 110°C for

90 min. A solvent mixture composed of chloroform:methanol:acetic acid:water, mixed in a ratio 50:37.5:3.5:2 (v/v/v/v) was used [35]. It allowed separating Sms, which is the most slowly migrating fraction ($R_f = 0.00297$) from other lipids ($R_f = 0.00840$ or more), which migrated markedly faster.

Ceramides. The lipid-containing extracts were submitted to thin-layer chromatography on commercially available plates covered with silica gel (Merck). A solvent mixture composed of chloroform:methanol:25% ammonia, mixed in a ratio 20:5:0.2 (v/v/v) was used. The chromatography was continued until the front of solvent reached one third of the plate height. The plate was dried at room temperature and the chromatography was continued with a solvent mixture composed of heptane:isopropyl ether:acetic acid, mixed in a ratio 60:40:3 (v/v/v) [36, 37]. It allowed the separation of Cers ($R_f = 0.197$) from other lipids, which demonstrated both lower ($R_f = 0.162$ or less) and higher motilities ($R_f = 0.232$ or more). Standards of Cers: saturated FA Cer (C 2137, Sigma) and N-oleoyl-D-sphingosine (O 2386, Sigma) were used to localize the ceramide fraction on the chromatogram.

FA Assay in Sms and Cers

Sms and Cers were eluted from the gel and hydrolysed in 2 M KOH according to Engelmann et al. [38]. *p*-Bromophenacyl esters of released FA were prepared as described by Aoyama and Sato [39]. Separation and determination of those derivatives were performed by reversed-phase high-performance liquid chromatography (LaChrom System, Merck Hitachi) [40], which makes it possible to separate, identify and determine individual FAs in the investigated material. A representative FA chromatogram is presented in figure 1.

Since each mole of Sm and each mole of Cer contains one mole of FA, the molar amounts of FAs isolated from these sphingolipid fractions were treated as molar amounts of Sms and Cers, respectively.

Fig. 2. Total, SAFA, MUFA and PUFA contents in Sms of control (n = 10) and pre-eclamptic (n = 10) Wharton's jelly. *** p < 0.001.

Fig. 3. Total, SAFA, MUFA and PUFA contents in Cers of control (n = 10) and pre-eclamptic (n = 10) Wharton's jelly. *** p < 0.001.

Sphingoid Bases: Isolation and Assay

Sphingoid bases (sphingosine, sphinganine, sphingosine 1-phosphate, sphinganine 1-phosphate, 4-hydroxysphinganine, galactosylsphingosine and glucosylsphingosine) were isolated from the above-described lipid-containing extracts by the method of Merrill et al. [41] with a slight modification. The lipids were submitted to solid-phase extraction with a 500-mg LC-18 column (Supelco). Sphingoids were eluted with 4 ml of methanol:10 mM triethylamine phosphate buffer (pH 7.2), mixed in a ratio 9:1 (v/v), and reacted with *o*-phthalaldehyde [41]. The resulting products were analysed by high-performance liquid chromatography using a C18 reversed-phase column (Chromolith RP-18e 100-4.6 mm, Merck). The solvent mixture contained methanol and 10 mM triethylamine phosphate buffer (pH 5.5) in the ratio 84:16 (v/v). This procedure separated the sphingoids, which eluted in the following order: sphingosine 1-phosphate, 4-hydroxysphinganine, sphinganine 1-phosphate, galactosylsphingosine with glucosylsphingosine, sphingosine and sphinganine.

Statistical Analysis

Mean $\pm$ standard deviations (SD) from 10 assays were calculated. Individual FA content in Sms and Cers was expressed in nanomoles per gram of fresh tissue. The results were submitted to statistical analysis by Student's t test, accepting p < 0.05 as significant.

Results

Sphingomyelins

Figure 2 shows that the Wharton's jelly of normal newborns contained above 6 μ mol of Sms per gram of tissue. Most of them (about 5 μ mol) contained saturated FAs

(SAFAs). The amounts of those containing monounsaturated FAs (MUFAs) and polyunsaturated FAs (PUFAs) were distinctly lower – about 1.2 and 0.7 μ mol/g, respectively.

Table 1 presents the individual FA contents of Sms of Wharton's jelly. It is apparent that stearate (C_{18:0}) and arachidate (C_{20:0}) were the most abundant SAFAs. They constituted above 60% of total Sm-bound FAs. The other SAFAs constituted about 8% of all FAs contained in this sphingolipid fraction.

The amount of MUFA-containing Sms was rather low: about 16% of total Sms. Most of them contained myristoleate (C_{14:1}) and oleate (C_{18:1}). Very low quantities of other MUFAs (C_{16:1} and C_{24:1}) were found (table 1).

The amounts of PUFA-containing Sms were lower than those which contained MUFAs. C_{18:2} and C_{20:4} were the main FA constituents of this fraction. Furthermore, a few percent of C_{18:3}, C_{20:5} and C_{22:6} were detected as well (table 1).

Pre-eclampsia was accompanied by an approximately 35% increase in the quantity of total Sms. Since the amount of SAFA-containing Sms does not change, the elevation of total Sm content results from an increase in MUFA- and PUFA-containing Sms (fig. 2).

Furthermore, significant changes in proportional amounts of individual Sms were observed. First of all, an increase in the quantities of those containing C_{12:0}, C_{14:1} and C_{20:5} was found. A marked decrease in Sms containing C_{18:0}, C_{20:0} and C_{18:1} was observed (table 1).

Table 1. FA content in Sms of control (n = 10) and pre-eclamptic (n = 10) Wharton's jelly

FA	Content, nmol/g tissue		Content, moles/1,000 mol of total FA	
	control	pre-eclamptic	control	pre-eclamptic
SAFAs				
C _{12:0}	463.96 ± 92.67	3,062.78 ± 102.91***	67.4 ± 12.1	328.9 ± 14.3***
C _{14:0}	119.22 ± 8.12	35.04 ± 7.23***	17.3 ± 2.5	12.5 ± 2.3***
C _{16:0}	65.37 ± 4.42	17.01 ± 3.53***	9.5 ± 1.6	6.1 ± 1.3***
C _{18:0}	2,218.67 ± 84.23	359.36 ± 72.78***	322.2 ± 23.97	128.6 ± 21.24***
C _{20:0}	2,074.24 ± 65.55	259.96 ± 57.36***	301.2 ± 3.6	93.1 ± 2.9***
C _{22:0}	11.48 ± 0.25	0.73 ± 0.21***	1.7 ± 0.2	0.3 ± 0.1***
MUFAs				
C _{14:1}	618.80 ± 123.27	2,583.24 ± 132.84***	89.8 ± 16.5	277.4 ± 18.2***
C _{16:1}	137.01 ± 24.29	96.98 ± 20.08*	19.9 ± 2.4	10.4 ± 2.1***
C _{18:1}	412.33 ± 14.58	58.20 ± 12.37***	59.9 ± 2.3	6.2 ± 1.7***
C _{24:1}	11.47 ± 0.78	2.44 ± 0.57***	1.7 ± 0.2	0.3 ± 0.1***
PUFAs				
C _{18:2}	278.45 ± 45.67	204.80 ± 40.92**	40.4 ± 4.8	22.0 ± 4.4***
C _{18:3}	124.41 ± 23.34	166.81 ± 26.56**	18.1 ± 3.5	17.9 ± 3.4
C _{20:4}	214.61 ± 41.89	224.92 ± 42.17	31.2 ± 5.6	24.2 ± 4.8*
C _{20:5}	61.68 ± 13.02	598.04 ± 15.75***	9.0 ± 1.7	64.2 ± 2.2***
C _{22:6}	75.16 ± 12.81	72.73 ± 12.23	10.9 ± 1.8	7.8 ± 1.6**

* p < 0.05, ** p < 0.01, *** p < 0.001.

Table 2. FA content in Cers of control (n = 10) and pre-eclamptic (n = 10) Wharton's jelly

Fatty acid	Content, nmol/g tissue		Content, moles/1,000 mol of total FA	
	control	pre-eclampsia	control	pre-eclampsia
SAFAs				
C _{12:0}	236.00 ± 42.54	734.66 ± 44.92***	91.6 ± 20.1	120.6 ± 21.6**
C _{14:0}	42.99 ± 8.43	undetectable	16.7 ± 4.0	0
C _{16:0}	37.55 ± 4.66	19.88 ± 4.07***	14.6 ± 1.2	3.3 ± 0.8***
C _{18:0}	900.52 ± 179.78	1,051.43 ± 185.73	349.6 ± 37.1	172.5 ± 35.2***
C _{20:0}	78.66 ± 15.89	397.23 ± 17.18***	30.5 ± 6.2	65.2 ± 6.8***
C _{22:0}	4.72 ± 1.09	9.33 ± 1.38***	1.8 ± 0.5	1.5 ± 0.4
MUFAs				
C _{14:1}	199.47 ± 39.27	2,396.64 ± 41.96***	77.4 ± 15.3	393.3 ± 16.1***
C _{16:1}	66.42 ± 14.76	84.85 ± 15.58*	25.8 ± 3.1	13.9 ± 2.8***
C _{18:1}	137.97 ± 28.65	374.50 ± 30.72***	53.6 ± 10.6	61.5 ± 11.2
C _{24:1}	4.73 ± 1.89	19.55 ± 2.29***	1.8 ± 0.4	3.2 ± 0.5***
PUFAs				
C _{18:2}	79.64 ± 3.48	17.38 ± 3.25***	30.9 ± 0.8	2.8 ± 0.6***
C _{18:3}	64.11 ± 8.29	38.64 ± 7.33***	24.9 ± 1.5	6.3 ± 1.3***
C _{20:4}	57.96 ± 11.41	60.52 ± 12.07	22.5 ± 2.2	9.9 ± 2.0***
C _{20:5}	516.30 ± 107.39	814.17 ± 116.94***	200.4 ± 29.7	133.6 ± 28.9***
C _{22:6}	158.15 ± 14.83	74.50 ± 13.89***	61.4 ± 2.9	12.2 ± 2.6***

* p < 0.05, ** p < 0.01, *** p < 0.001.

Fig. 4. Sphingoid base contents in control (n = 10) and pre-eclamptic (n = 10) Wharton's jelly. Spho = Sphingosine; Spha = sphinganine; 4-OH-Spha = 4-OH-sphinganine; Spho-1-P = sphingosine 1-phosphate; Spha-1-P = sphinganine 1-phosphate; Gal-Spho + Glu-Spho = galactosyl- and glucosyl-sphingosine. ** p < 0.01, *** p < 0.001.

Ceramides

Figure 3 shows that Wharton's jelly contained about 2.6 μmol of Cers per gram of tissue. The amounts of SAFA-containing Cers and those containing unsaturated FAs (MUFAs + PUFAs) were similar. It is worthy of note that the quantity of PUFA-containing Cers was twice as high as that of MUFA-containing Cers.

Table 2 presents the individual Cer-bound FA contents in Wharton's jelly. As in Sms stearate ($\text{C}_{18:0}$) was the most abundant both in total Cers and in the SAFA-containing Cer fraction. It constituted almost 35% of Cer-bound FAs. Furthermore, a high amount of $\text{C}_{20:5}$ (20%) was detected. Distinctly lower amounts of other Cer-bound FAs were detected. Their individual quantities varied from 1.8 to 9.2%.

Pre-eclampsia was associated with a significant increase in total Cer content in Wharton's jelly, mainly those which contain MUFAs and SAFAs. The MUFA-containing Cers became a dominating fraction (fig. 3).

Significant alterations in the proportional amounts of individual Cer-bound FAs were detected. A distinct increase in $\text{C}_{14:1}$ and $\text{C}_{20:0}$ and $\text{C}_{12:0}$ was accompanied by a reduction in other FA-containing Cers, especially those which contain $\text{C}_{18:2}$ and $\text{C}_{22:6}$ (table 2).

Other Sphingoids

Figure 4 shows that the amounts of sphingosine, sphinganine and their simple derivatives were distinctly lower compared to those of Sms and Cers. Only nanomole amounts of these substances were found. Free sphinga-

nine, sphingosine and sphinganine 1-phosphate were the predominating compounds. Only trace amounts of glycosylated sphingoid bases were detected.

Pre-eclampsia was associated with significant alterations in free sphingoid bases and their simple derivatives in Wharton's jelly. First of all, a distinct increase in sphinganine, sphingosine and sphingosine 1-phosphate was found. This was accompanied with a marked decrease in sphinganine 1-phosphate (fig. 4).

Molar Ratio of Various Sphingoids

As can be seen from figure 5, the Sm:Cer ratio of control Wharton's jelly approximates 2.66 whereas in pre-eclamptic material it reached a markedly lower value (about 1.53). Also a marked decrease in the ratio of phosphorylated sphinganine to phosphorylated sphingosine was found (from 3.01 in control to 1.06 in pre-eclamptic material). In contrast, the ratio of free sphinganine to free sphingosine increased from 2.23 in control to 2.72 in pre-eclamptic material (fig. 5).

Discussion

Sphingolipids, including Sms, Cers and some sphingoid bases, are bioactive molecules which contribute to the regulation of signal transduction pathways, direction of protein sorting and the mediation of cell-to-cell interactions and recognition. They have also been reported to cluster with sterols to form lipid microdomains (rafts),

Fig. 5. Molar ratio of selected sphingoids in control (n = 10) and pre-eclamptic (n = 10) Wharton's jelly. Sm:Cer = Sm to Cer ratio; Spha:Spho = sphinganine to sphingosine ratio; Spha-1-P:Spho-1-P = sphinganine 1-phosphate to sphingosine 1-phosphate ratio. * p < 0.05, *** p < 0.001.

which play an important role in biomembrane formation [42].

Sphinganine, a member of the sphingoid base family, is a metabolite which participates in the early steps of Sm formation. At the first step serine is condensed with palmitoyl residue (from palmitoyl-CoA) by serine palmitoyl transferase to generate 3-keto-dihydrosphingosine with a release of CO₂. The main product of this reaction is subsequently reduced to form sphinganine (dihydrosphingosine), which binds FA residue by an amide bond converting to dihydroceramide [28]. Under the action dihydroceramide desaturase it is transformed into Cer, which serves as a substrate for Sm synthesis. Sm synthase 1 (in the Golgi apparatus) and Sm synthase 2 (in the plasma membrane) transfer the phosphorylated choline from phosphatidylcholine to the 1-hydroxyl group of Cer forming Sm [30]. They are strongly heterogeneous due to various FAs bound to the amino group of sphingosine.

Recently, we have evaluated the sphingolipid components of the UCAs [43] and UCVs [unpubl. observations] in healthy newborns and those delivered by pre-eclamptic mothers. The walls of both UC vessels are abundant in Sm and Cer, whereas the amounts of sphingoid bases are rather low. Pre-eclampsia is associated with significant increases in Sm and sphinganine with a decrease in Cers and other sphingoid bases in UCA walls [43]. In con-

trast, a significant decrease in both Sms and Cers in UCV was observed [unpubl. observations].

Here we have evaluated the sphingolipids of Wharton's jelly and their alterations in pre-eclampsia. This study shows that Wharton's jelly contains numerous sphingolipids. The Sms and Cers exist in significant, micromole amounts per gram of fresh tissue. The molecular weight of C₂₄ FAs is almost twice as high as that of C₁₂ FAs. For this reason, the amounts of individual FA moles/gram of tissue distinctly differs from the amounts of individual FA moles/1,000 moles of total FAs. In both Sms and Cers of control Wharton's jelly, the predominating FA is stearate (C_{18:0}). The contents of sphingoid bases and their phospho- and glycoderivatives are distinctly lower: only nanomole amounts per gram of fresh tissue were found.

Pre-eclampsia was accompanied by a significant alteration in sphingolipid composition. First of all, a distinct increase in Cer content was observed. The amount of Sm grew to a lesser degree. For this reason, the Sm:Cer ratio markedly decreased. Only a slight increase in the ratio of free sphinganine (biosynthetic precursor of Sm) to free sphingosine (Sm degradation product) was found in pre-eclamptic tissue. In contrast, the proportional amounts of phosphorylated forms of these bases changed in the opposite direction. The ratio of sphinganine 1-phosphate to sphingosine 1-phosphate in pre-eclamptic Wharton's jelly decreased 3 times in comparison to control material.

The increase of sphingolipids in pre-eclamptic tissue may be the result of increased synthesis, decreased degradation or coexistence of both processes.

It was observed in our recent study [44] that pre-eclampsia is associated with a slight increase in total FA content in Wharton's jelly and with marked changes in the proportional relationships between various lipids. A distinct decrease in the amounts of free FAs was observed with a concomitant increase in monoacylglycerols and cholesterol esters.

Sms may serve as source of secondary messengers, which participate in signal transduction from cell membrane to cytoplasm. Binding of some cytokines (e.g. interferons or tumour necrosis factor) by their receptors results in the stimulation of Mg²⁺-dependent neutral sphingomyelinase, which cleaves Sms with a release of phosphorylated choline and Cers. The latter starts the Sm signal transduction pathway inside the cell [45]. Cers can evoke membrane structure changes, and activate some enzymes, like protein kinase C, protein phosphatases 1 and 2A, cathepsin D and c-Raf [30] and thus perform

critical functions for cell growth, survival and apoptosis [46].

Furthermore, Cers may serve as substrates for resynthesis of Sms. The distinct increase in Cer content in pre-eclamptic Wharton's jelly may enhance Sm synthesis in this tissue.

Sphingosine is a product of the enzymatic hydrolysis of Cers. It becomes engaged in the modulation of protein kinases [47], phospholipase C [48] and other signaling pathways, inducing the arrest of cell division or apoptosis [47]. The marked increase of sphingosine in Wharton's jelly of pre-eclamptic subjects may enhance these processes.

Free (non-phosphorylated) sphingosine and sphinganine are the only sphingoids, which easily penetrate all biological membranes [49]. They can be exchanged between cells and the extracellular matrix or between tissues and the circulating blood [50].

Sphingosine and sphinganine are phosphorylated by one of two sphingosine kinases. Both sphingosine 1-phosphate and sphinganine 1-phosphate act through cell surface G-protein-coupled receptors, which are coded by the endothelial differentiation gene family, thus regulating diverse G proteins and subsequently various intracellular signaling pathways [51].

Sphingosine 1-phosphate is a tumour-promoting lipid involved in the regulation of proliferation, cell growth, cell survival, cell migration, inflammation, angiogenesis, vasculogenesis and resistance to apoptosis. It shows antagonizing effects to those exerted by Cers [52]. It is of interest that both sphingosine 1-phosphate and sphinganine 1-phosphate bind to the same receptors, but their effects are distinctly different. The effects exerted by sphingosine 1-phosphate are 20–50 times stronger than those exerted by sphinganine 1-phosphate [53]. The increased amount of sphingosine 1-phosphate with a simultaneous increase in Cer content suggests a change in intracellular signal transduction in pre-eclamptic tissue.

We also detected some other sphingoid bases and their glycosylated derivatives: 4-OH-sphinganine, Glc-sphingosine and Gal-sphingosine, but their amounts were very low. It is known that the glycosylated sphingoid bases are cytotoxic [54], but we doubt whether a trace amount of these substances exerts such an effect on the cells of Wharton's jelly.

It is apparent from our study that pre-eclampsia is associated with distinct alterations in the Sm, Cer and sphingoid base composition of Wharton's jelly. A marked increase in Sm, Cer, sphingosine, sphinganine and sphingosine 1-phosphate accompanied by a decrease in sphin-

ganine 1-phosphate were observed. Since some of them, especially Cers, sphingosine and sphingosine 1-phosphate, play a role of secondary messengers in intracellular signal transduction, these alterations may modify intracellular metabolism and may result in the change of lipid compounds described in our recent paper [44].

Furthermore, Sms play a part in the formation of a lipid bilayer in the cell membranes. Together with free cholesterol, they form so-called rafts in the outer layer of the plasma membrane. Those rafts agglomerate different receptors [55–57] or they are the place where receptors are directed after ligand binding and can start next steps of signal transduction [58–60]. Proper fluidity of plasma membrane, which depends mainly on hydrophobic interactions between hydrocarbon chains of phospholipids and cholesterol rings, is required for these functions.

Pre-eclampsia-induced increase in Sm content may enhance second-messenger production, and may increase the number and size of lipid rafts in the cell membranes, resulting in intensification of the signal transduction system within the cells. At least in some cases, the effects exerted by PGFs are mediated by the lipid second messengers. Thus alterations in lipid compounds of Wharton's jelly may participate in the deregulation of various cell functions, including lipid metabolism [44] and overproduction of sulphated glycosaminoglycans or down-regulation of enzymes which participate in their degradation [1, 15].

Sms as membrane compounds are not themselves engaged in cell regulation, but they are an abundant source of second messengers, mainly Cers, sphingosine and sphingosine 1-phosphate [28, 29, 31]. Their role in the biosynthesis of ECM was not extensively studied. However, it was reported that Cers and free sphingoid bases exert inhibitory effects on cell division [61]. It has been known for many years that fibroblasts in an exponential growth phase (in vitro) produce low amounts of ECM whereas those in the phase of contact inhibition synthesize distinctly more of these compounds [62].

Significant increases Cers and free sphingoid bases in pre-eclamptic Wharton's jelly may inhibit cell division in this tissue and stimulate them to produce more extracellular components. It was reported by Gilbert et al. [63] that sphingomyelinase – an enzyme which converts Sms into Cers and phosphocholine – increased the release of newly synthesized sulphated glycosaminoglycans and collagen into the media by primary articular chondrocytes in vitro. They suggest that even small increases in endogenous Cers in the cells tilt the homeostatic balance toward ECM synthesis [64]. Furthermore, Sato et al. [64]

demonstrated that endogenous Cer is a positive regulator of collagen gene expression. They found that the amount of this second messenger increases under the action of TGF- β .

It was reported in our previous paper [65] that Wharton's jelly contains large amount of TGF- β (above 7 times more than the UCA wall) and distinctly more collagen [5] in comparison to the walls of the UC vessels [18, 19, 22]. It seems that strongly TGF- β -stimulated myofibroblasts

of Wharton's jelly produce more Cers which stimulate them to synthesize more collagen than the cells contained in other parts of the UC.

It may be suggested that high amounts of Sm degradation products, which act as second messengers in signal transduction, play an important (if not a main) role in stimulating scanty cells of Wharton's jelly to produce large amounts of ECM components and in remodelling this tissue in pre-eclampsia.

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