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Differences of glycocalyx composition in the structural elements of placenta in preeclampsia

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ABSTRACT

Introduction: Glycans expressed in the fetal-maternal interface were shown to exert immunomodulating effects and to mediate interactions between the cells. The aim of this study was to investigate alterations in the structure of carbohydrate chains of glycocalyx in placental tissue in pregnancies complicated with preeclampsia (PE).
Methods: A histochemical analysis of placental tissues was performed with a panel of biotinylated lectins. We analyzed placental tissues in women who had severe or moderate PE and compared them to placentas from women with normal pregnancies.
Results: There was decreased content of terminal residues of α(2,6)-linked sialic acid (as stained by SNA lectin) in the carbohydrate chains of glycocalyx of the endothelium of placental terminal villi in patients with moderate preeclampsia. The composition of the glycocalyx of syncytiotrophoblast in patients of this group did not differ from the control group. Amount of the glycans with terminal β-Gal- (ECL) and α-mannosyl residues (ConA) in the syncytiotrophoblast and capillary endothelium of the placenta was significantly higher in the group with severe PE compared to the control group. The increased content of sialoglycans with α(2,6)-linked sialic acids residues were discovered in the syncytium, and the decreased content of α(2,3)-linked sialic acids residues – in the endothelium of terminal villi in preeclampsia.
Discussion: The most prominent alteration of the glycocalyx composition was found in the placentas of women with severe preeclampsia. It is likely that the modified glycome of syncytiotrophoblast and capillary endothelium may play an important role in pathogenesis of preeclampsia.

1. Introduction

Preeclampsia (PE) is a pregnancy specific syndrome that may manifest after 20 weeks of gestation and is characterized by high maternal and perinatal morbidity and mortality [1]. Although much research on the mechanism of preeclampsia has been performed, there is no consensus about its etiology and pathogenesis. It is thought that some extragenital maternal pathologies cause utero-

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placental blood flow insufficiency and therefore result in poor trophoblast invasion into the spiral arteries [2,3]. Currently, there are several models of the interactions between maternal and fetal cells, with endothelial mimicry being the most common. An alternative concept is the lectin-carbohydrate interaction model [4], which is supported by recent data on the glycosylation of α5β1 integrin, a key molecule in the intercellular interactions [5,6].

Various carbohydrate-binding proteins and carbohydrate ligands have been found on the cell surface of the fetoplacental system. This suggests that the lectin-carbohydrate interaction may play an important role in placentation and angiogenesis [7–9]. The carbohydrate profile of placentas from women with normal pregnancies showed that all placental structures (cytotrophoblast, syncytiotrophoblast, stroma, endothelial cells, Kashchenko-Hofbauer cells, etc.) contain carbohydrate residues [10]. The analysis of glycan distribution in the placentas of women with complicated pregnancies (hypertensive disorders, PE, etc.) revealed that tissues of placental structures with morphological and functional abnormalities have a modified carbohydrate profile [11]. Additionally, there is no detailed information about the effect of PE severity on the placental carbohydrate profile.

In this study we have focused on the profiled glycans forming glycocalyx of the syncytiotrophoblast and endothelium of the terminal placental villi. Composition of the glycocalyx can be considered as a "carbohydrate phenotype" (a combination of cell surface glycans) which can change under different pathologic conditions and may have clinical significance. Interest to the glycocalyx is substantiated by its key role in intercellular interactions during the invasion and immune response [12], assuming its contribution to the pathogenesis of preeclampsia. The aim of our work was to study alterations in the composition of carbohydrate chains of glycocalyx in the structural elements of placenta from women with moderate or severe preeclampsia.

2. Materials and methods

The research protocol was approved by the Institutional Review Board at the Research Center for Obstetrics, Gynecology and Perinatology.

We analyzed placental tissue samples, clinical data and history of 23 women. The PE group (n = 16) included 20–40 year old patients with a natural singleton pregnancy complicated by preeclampsia. The control group (n = 7) included 20–40 year old women with normal natural singleton pregnancy (*N*P-group). PE group was divided into two subgroups. The PE-1 group (n = 10) included patients with moderate PE (urine protein level range between 0.3 and 0.5 g/day, arterial blood pressure between 140/90 and 160/100 mm Hg). The PE-2 group (n = 6) included women with severe PE (urine protein level ≥ 0.5 g/day and arterial blood pressure ≥ 160/100 mm Hg). All patients delivered by cesarean section. The clinical profile of all study groups is shown in Table 1.

The study of carbohydrate phenotype of placenta was carried out through the lectin staining of syncytiotrophoblast membranes and of membranes of endothelial cells of terminal placental villi. The samples of paracentral area of placenta first underwent routine morphologic (macroscopic and histologic) examination to exclude presence of infarcts, hemorrhage and calcium deposits, and afterwards were submitted for histochemical investigation. The carbohydrate phenotype was analyzed with six biotinylated lectins of plant origin: MAL II, SNA, ECL, UEA I, Con A and VVL (Vector Labs, USA) (Table 2). The number of sections from a placental sample from each patient was 15: 6 sections incubated with 6 above mentioned lectins, 3 sections pretreated with neuraminidase prior to study of SNA, MAL II and ECL lectins binding, and 6 sections of negative control (1 per lectin).

The 4-μm thick tissue sections were mounted onto Superfrost Plus slides (Menzel-Glaser GmbH and Co, Germany). All of the lectins were prediluted in phosphate buffered saline (PBS, Sigma, USA) supplemented with 4% bovine serum albumin (BSA, PanEco, Russia) to achieve the final concentration of 10 μg/ml, that is consistent with the literature [13].

The carbohydrate specificity of the lectins is summarized in Table 2 [14].

The staining was performed according to a standard protocol. Briefly, the glycans were unmasked in citrate buffer (Spring Bioscience, USA) at pH 6.0 in a microwave oven at 600 Watts. Then, to block endogenous peroxidase activity, the sections were incubated for 10 min in a 3% H_2O_2 solution (Spring Bioscience, USA). The sections were washed in PBS (pH = 7.6) three times for 5 min each. According to the recommendations (Spring Bioscience, springbio.com) to block nonspecific staining, Protein Block (Spring Bioscience, USA) was used at room temperature for 10 min. Incubation of sections together with biotinylated lectins was performed overnight at 4°C according to dos-Santos [15].

After incubation with lectins each slide was washed with PBS three times, and the sections were incubated for 30 min at room temperature with a streptavidin-peroxidase conjugate (Amercham, USA) diluted in PBS 1:1000. The lectins were then removed and the sections were washed again (PBS, three times). For visualization of carbohydrate fragments we used diaminobenzidine (DAB) (Spring Bioscience, USA), containing DAB chromogen (3,3′ diaminobenzidine) which was diluted with a buffer – substrate (pH 7.5) containing hydrogen peroxide. For better visualization of the placental structures, the sections were additionally stained with Mayer's hematoxylin solution (BioVitrum, Russia) for 20 s.

Study of SNA, MAL II and ECL lectins binding was carried out before and after pretreatment with 50 mU/ml neuraminidase from *Vibrio cholerae* (Type III, buffered aqueous solution, sterile-filtered, 1–5 units/mg protein, Sigma-ALDRICH, USA). Neuraminidase pretreatment was used for 1 h at 37 °C, as described by Jones [13], to remove terminal sialyl residues from oligosaccharide chains.

As a negative control for each of the six conjugated lectins we used histochemical staining without adding lectins to incubation medium according to recommendation of Sgambatia [16].

As positive control we used the same mature placentas which were the subjects of present study.

Eclipse 80i microscope (Nikon Corporation, Japan) was used to examine the lectin staining. The intensity of staining was evaluated in the glycocalyx in 10 visual fields in each section. The measuring point (field) was selected by researcher manually. The evaluation of the intensity of reaction was conducted by two independent researchers "blindly" (the samples were encrypted). We used the NIS-Elements Advanced Research 3.2 program (Laboratory Imaging LTD, Czech Republic). For convenience, the obtained data were multiplied by 100 for the analysis.

Statistical analysis was performed with the software package "Statistica for Windows v. 8" (StatSoft Inc., USA). The Kolmogorov-Smirnov test was applied to analyze the distribution of input data. The mean values of variables, the standard errors, and the 95% confidence intervals were determined. Student's t-test and analysis of variance were used to compare the mean values. Differences were considered statistically significant at $p < 0.05$.

3. Results

The study of carbohydrate placental profile with a panel of biotinylated lectins showed that in the study groups the stained reaction product was visualized in endothelial cells of capillary endothelium and syncytiotrophoblast glycocalyx of the terminal villi, but with different intensity. In all groups the intensity of