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GLYCOSYLATION PATTERNS OF PLACENTAL PROTEINS IN BLIGHTED OVUM

GLIKOZILACIJA PROTEINA POSTELJICE U ANEMBRIONALNOJ TRUDNOĆI

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Original paper

Key words: glycoproteins, lectins, blighted ovum

SUMMARY. Blighted ovum is a form of miscarriage, involving the absence or disappearance of an embryo very early in pregnancy. Due to contemporary lack of understanding of the nature of blighted ovum, our objective was to demonstrate the glycosylation patterns of placental proteins in this failure of pregnancy. Six placentas were taken from women with blighted ovum and twentyone placentas were provided from healthy women undergoing elective termination of normal pregnancies. Olygosaccharide branches were detected by Western-blot method using lectins: SNA and PHA-E, after preliminary separation of proteins by discontinuous SDS-PAG electrophoresis. Much stronger expression of GP74 has been identified in blighted ovum at the beginning of the eleventh week of gestation (with PHA-E), than in normal placenta. The same result was obtained for GP25 being stronger in blighted ovum at the end of eleventh week (with SNA). It is possible to conclude that the differences in glycoprotein changes beetwen the blighted ovum and the normal placenta, can only be found at the quantitative level, but not at the qualitative level.

Izvorni članak

Cljučne riječi: glikoproteini, lektini, blighted ovum

SAŽETAK. »Blighted ovum« (anembrionalna trudnoća) predstavlja oblik pobačaja, koji uključuje nedostatak ili nestanak zametka vrlo rano tijekom trudnoće. Dosadašnja skromna saznanja o ovom poremećaju potaknula su nas na detaljnija ispitivanja prikaza glikozilacijskih obrazaca placentalnih proteina. Promjene glikoproteinskog sastava detektirane su Western-blot metodom koristeći lektine SNA i PHA-E, koja je slijedila nakon preliminarnu separacije proteina diskontinuiranom PAGE elektroforezom. Uspjeli smo identificirati mnogo jaču ekspresiju GP 74 u blighted ovum-u, nego u normalnoj placenti na početku jedanaestoga tjedna trudnoće, koristeći lektin PHA-E. Isti smo rezultat dobili i za GP25, koji je bio jačeg intenziteta u blighted ovum-u pri kraju jedanaestoga tjedna, koristeći lektin SNA. Naši rezultati navode nas na zaključak da se razlika između blighted ovum-a i normalne placente može uočiti samo na razini kvantitativnih glikoproteinskih promjena, ali ne i na kvalitativnoj razini.

Introduction

Blighted ovum (anembryonic pregnancy; AP) is a frequent presentation of first-trimester abortion and excluding those with chromosomal abnormalities, there remains a high percentage of abortion occurring without discernible causative mechanisms.¹ Recently, defective expression of some growth factors and/or cytokines was suggested to be causative for these reproductive failures.² All of these factors are glycoproteins (GP) biochemically speaking, and they have attracted impressive attention by many researchers in recent years. This has promoted universal re-evaluation of the importance of carbohydrates in biology and medicine.³ Since glycoproteins play such an important role in health and disease, we have studied the differences in glycoprotein patterns beetwen the blighted ovum and normal pregnancy.

The primary goal was to find differences in glycosylation patterns beetwen normal pregnancy and the

blighted ovum, hoping and trying to find the molecular differences between normal gestation and this failure of pregnancy, as the real problem.

Materials and methods

Materials

The following analytical reagents: 5-bromo-4-chloro-3-indolyl phosphate (BCIP), nitro blue tetrazolium (NBT), ammonium persulfate (APS), glycine, magnesium chloride, sodium chloride, sodium dodecyl sulfate and Trizma base, were all purchased from Sigma (St. Louis, MO); bovine serum albumin (BSA), glycerol, p-mercaptoethanol, phenylmethylsulfonyl fluoride, Rotiphorese gel 30 and Tween 20 were purchased from Roth (Karlsruhe); ethanol, hydrochloric acid, methanol and acetic acid from Kemika (Zagreb); N,N,N',N'-Tetramethylethylenediamine from Merck (Darmstadt); immobilion PVDF membrane from Millipore (Bedford, MA); digoxigenin-labeled SNA lectin and alkaline pho-

sphatase-conjugated antidigoxigenin Fab fragments from Boehringer Mannheim (Mannheim); biotin-labeled PHA-E, UEA-I and DBA lectins, streptavidin alkaline phosphatase conjugate, bromphenol blue and Coomassie brilliant blue R-250, from Serva (Heidelberg).

Placental samples. Tissue samples from 21 placentas were provided from healthy women undergoing elective termination of normal pregnancies between 7th and 17th weeks of gestation (control samples). The second group consisted of 6 pregnant women with blighted ovum diagnosed by the routine transvaginal ultrasound.

Methods

The operative method used was cervical dilatation, followed by vacuum extraction or curettage. Gestational ages were assessed by the date of the last menses and confirmed by ultrasound and clinical examination. Immediately after evacuation, placental tissues were frozen in liquid nitrogen and stored deeply frozen at -80°C until used.

Placental tissues were mechanically homogenised in homogenisation buffer (50 mM Tris HCl, pH 7,5; 100 mM NaCl; 1 mM EDTA) containing 1 mM phenylmethylsulphonyl fluoride. The homogenates were centrifuged at 5,000 g for 10 minutes and supernatants were stored at -80°C .

Polyacrylamide gel electrophoresis

Gel electrophoresis of glycoproteins was performed in 10% polyacrylamide gels containing 0,1% sodium dodecylsulfate (SDS) according to Laemmli⁴ (SDS-PAGE). Protein samples were dissolved in sample buffer (pH 6,8; Tris HCl 50 mM, Glycerol 10%, β -mercaptoethanol 5%, SDS 2%, Bromphenol-blue 0,1% in distilled H₂O) in the ratio 1:1. They were denaturated for 5 minutes at 95°C before use. Aliquots of 20 μg of total proteins were applied to each slot and after migration of the samples at 80 V from the stacking gel into the separation gel, proteins were finally separated at 120 V.

Western blotting

After electrophoresis, proteins were transferred to the PVDF immobilion membrane in a semidry blotting system (Pharmacia, Sweden) with the semidry buffer (Tris HCl 48 mM, Glycin 39 mM; SDS 1,4 mM; methanol 20%).⁵ Blotting was carried out at 0,8 mA/cm² over a period of 60 minutes. After blotting, the part of PVDF membrane with standard proteins was separated, and the rest of the membrane was blocked overnight with 3% bovine serum albumin (pH 7,5).

Identification of proteins after blotting

After overnight blocking, the membrane was incubated with lectins in the lectin buffer (MgCl₂ 1mM; pH 7,5; CaCl₂ 1mM; in TBS). Lectin-glycoprotein complexes were detected with antidigoxigenin Fab fragments conjugated to alkaline phosphatase (for lectin

SNA), or streptavidin-alkaline phosphatase conjugate (for lectin PHA-E), and visualized with BCIP (5-bromo-chloro-3-indolyl phosphate)/NBT (nitro blue tetrazolium).^{6,7}

Results

Detection of placental glycoproteins in blighted ovum by lectin SNA

Lectin SNA used for identification of glycoproteins from the blighted ovum from the eleventh week of gestation provided results presented in *Figure 1*.

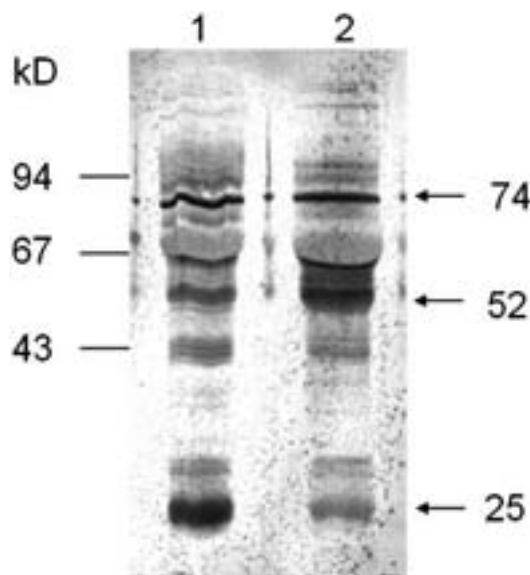


Figure 1. Placental glycoprotein pattern from the blighted ovum obtained by electrophoresis and detection with SNA from the eleventh week of pathological pregnancy (1) in comparison with the glycoprotein pattern of control placenta from the normal pregnancy (2) of the same gestational age

Slika 1. Prikaz elektroforetski razdvojenih glikoproteina posteljice dobivenih detekcijom lektinom SNA u patološkoj trudnoći (1) te u kontrolnoj posteljici (2) iste gestacijske starosti

The glycoprotein with the molecular weight 25 kD is more abundant in the pathological pattern than in the pattern of normal placenta of the same gestational age. At the same time the glycoproteins GP 74, GP 61, and GP 52 are equally abundant in both samples as visualized by their SNA-patterns. This constancy in the two patterns facilitates identification of the variable components.

Detection by lectin PHA-E

The glycoprotein with the Mw of 74 kD identified by PHA-E is more abundant in the pathological pattern than in the normal pregnancy pattern, while at the same time, glycoproteins GP 61 and GP 52 are equally abundant in both pathological and normal samples (*Figure 2*). They are again excellent constant components in both glycoprotein patterns which render additional credibility to our efforts and results on glycoprotein pattern comparison.

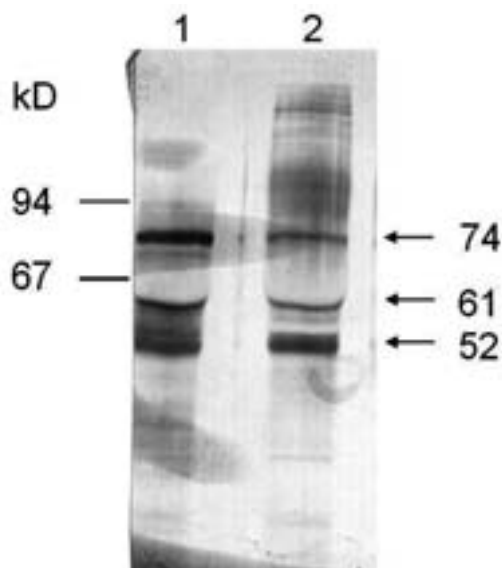


Figure 2. Placental glycoprotein pattern of the blighted ovum obtained by electrophoresis and detection with PHA-E lectin at the beginning of the eleventh week of pathological pregnancy (1) in comparison with the pattern of control placenta from the normal pregnancy (2) of the same gestational age

Slika 2. Prikaz elektroforetski razdvojenih glikoproteina posteljice dobitvenih detekcijom lektinom PHA-E u patološkoj trudnoći (1) te u kontrolnoj posteljici (2) iste gestacijske starosti

Discussion

Blighted ovum is a form of miscarriage, which involves absence or disappearance of human embryo very early in pregnancy. Aydin and collaborators have found defective chorionic villus vascularization associated with embryonic death and thus supported the hypothesis that anembryonic pregnancy resulted from early embryonic death and subsequent resorption of embryo, rather than from the non-development of embryo.⁸ Low levels of adenosine deaminase (an important enzyme in the degradative pathway of purine nucleotides) have been found in serum and placental tissues from patients with anembryonic pregnancies, which may lead to accumulation of products, toxic to DNA and causative for subsequent loss of the pregnancy.⁹ A defective chorionic villus vascularization, demonstrating inadequate vasculogenesis and abnormal development of the vasculosyncytial membrane, is seen in pregnancies complicated by embryonic death, and even more pronounced in the anembryonic pregnancies.¹⁰

Successful implantation and placentation is prerequisite for normal development of embryo, and obstructions at this stage could be the basis for many diseases related to pregnancy.¹¹ Results were obtained in experimental animals which indicate to the very glycoproteins to be the potential key molecules in intercellular interactions which bring about successful implantation. Hence we have concentrated our attention on glycoprotein changes in the anembryonic pregnancy.

Due to the contemporary lack of understanding of blighted ovum, we have demonstrated the glycosylation patterns of placental proteins in this failure of pregnancy for the first time.

The analyses carried out draw the conclusion that some glycoproteins, namely GP 61, GP 52 and GP 30, are present with roughly equal and constant intensities in the blighted ovum and in the normal pregnancy. Furthermore, we have identified much stronger expression of GP 74 at the beginning of the eleventh week of gestation (detected by lectin PHA-E) in the blighted ovum than in the normal placenta. The same result was obtained for GP 25 at the end of the eleventh week (detected by lectin SNA).

The presence of numerous differences in glycoprotein patterns indicates the connection between the developmental errors and protein glycosylation. Constant glycoprotein components in all of these patterns offered for comparison and obtained both by SNA and PHA-E, prove the credibility of the applied method and enable the comparison of the variable components in the respective patterns.

The final conclusion reached is that the differences between the blighted ovum and the normal placenta can only be found at the quantitative level of glycoprotein changes, but not at the qualitative level.

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