Thrombomodulin in human gestational tissues: placenta, fetal membranes and myometrium

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Abstract

Purpose: In intervillous space, thrombomodulin (TM) can be a key component of the protein C anticoagulant pathway that controls the balance between coagulation and anticoagulation/ /fibrinolysis via activation of protein C (APC). In our working hypothesis we assume that not only TM from the placenta, but also TM from myometrium might be engaged in this protective mechanism. To determine whether this is potentially possible, we decided to measure TM concentration in placenta and myometrium, and also in fetal membranes.

Material and methods: The study group consisted of 35 parturient women who delivered at term by cesarean section. Strips of placenta, fetal membranes and myometrium, as well as venous blood samples were collected during operation. The tissues were homogenized. TM was measured by immunoenzymatic method (ELISA).The concentration of TM antigen in placenta was 18.76±3.83 ng/mg proteins, in fetal membranes 8.57±1.64 ng/mg proteins and in myometrium 4.72±1.93 ng/mg proteins, while in blood plasma it was 0.063±0.016 ng/mg proteins.

Conclusions: It was shown for the first time that thrombomodulin is present in gestational myometrium and fetal membranes. The results support the hypothesis that not only placental TM, but also myometrial TM can participate in maintaining the fluidity of the blood in utero-placental circulation.

Key words: thrombomodulin, placenta, fetal membranes, myometrium.

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Introduction

Thrombomodulin (TM) is an integral membrane-glycoprotein of 75 kDa of molecular weight which is expressed on the capillary endothelium and therefore its highest concentration is found in densely vascularized organs like heart, lungs and placenta [1]. In placenta, TM is expressed by extravascular and vascular trophoblast as well as by fetal capilary endothelium [2].

TM is a high-affinity receptor for thrombin forming thrombin-thrombomodulin complexes which then activate zymogen protein C to a powerful anticoagulant protein C (APC). Thrombin-thrombomodulin complex can also activate the latent inhibitor of fibrinolysis, e.g. procarboxypeptidase B to carboxypeptidase B producing thrombin activatable fibrinolysis inhibitor (TAFI) [3,4]. Once APC is generated, it binds protein S as a cofactor and can then inactivate factors Va and VIIIa, thus decreasing thrombin generation. The rate of protein C activation by the thrombomodulin-thrombin complex is greatly enhanced when protein C is bound to the endothelial protein C receptor (EPCR) [5,6]. Furthermore, the fibrinolytic effect of TM *in vivo* depends on its concentration in local vasculature [7].

There are two main forms of TM which differ with respect to their composition: (I) cellular TM and (II) soluble TM (sTM), a proteolitically cleaved fragment of cellular TM which shows main properties of cellular TM [8]. Cellular TM consists of three portions – the extramembrane, transmembrane and intracellular. The extramembrane portion consists of three domains: N-terminal lecitin-like domain (D1) in which antiinflammatory properties of TM are contained, followed by EGF-like domain (D2) that can bind thrombin, and an O-glycosylation-rich domain (D3) [9].

sTM exists in plasma and urine [10], and in amniotic fluid [unpublished data]. It is often used as a marker of endothelial injury in various clinical settings: in disseminated intravascular coagulation (DIC), myocardial infarction, venous thromboembolism and others [8,11]. A significant increase of sTM was observed in preeclampsia [12] and pregnancy induced hyperten-

sion (PIH) [13], as well as in acute placental abruption [14], but not in recurrent pregnancy loss [15].

It has been suggested that TM is a key component in control of the fluidity of blood in the intervillous space and therefore protects the utero-placental circulation against local thrombosis (placental thrombosis) [16]. In our working hypothesis we assume that not only TM from the placenta, but also TM from myometrium, a highly vascularized tissue, can induce locally APC production and thus prevent the utero-placental circulation from hypercoagulability as well. To determine whether this is potentially possible, we decided to measure TM concentration in placenta and myometrium, and also in fetal membranes.

Material and methods

Patients

The study group consisted of 35 parturient women (23.8±3.1 of age), 21 primapares and 14 multipares, with a normal course of pregnancy (we excluded from our analysis complicated pregnancy, such as placenta previa and low-lying placenta, as well as placental abruption, preeclampsia, prolonged rupture of membranes and intraamniotic infection). Indications for cesarean section were as follows: fetal distress in a subgroup of labouring patients – 7; a subgroup of elective sections: patients with two cesarean births in anamnesis – 4, with more than three cesarean births – 3; breech presentation with a primapara – 5; breech presentation with a patient after a cesarean section – 4; transversal position – 2.

The control group consisted of 20 healthy, nonpregnant women, 20-25 years old, in the luteal phase of menstrual cycle.

All women were informed about the research and they accepted the sampling of placenta, myometrium, and blood. Permission of the Bioethics Committee was also obtained.

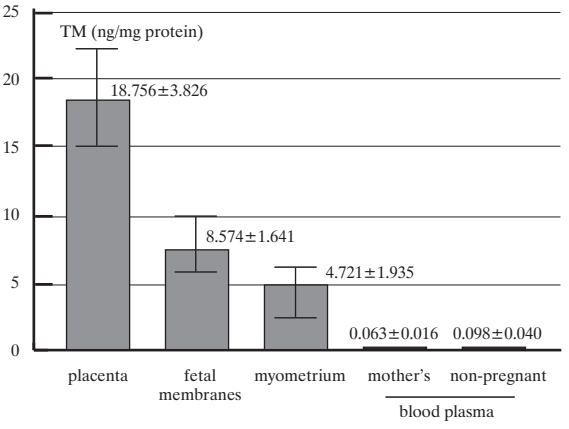
Sampling of the material

All materials were obtained at the time of the caesarean section: (I) strips of myometrium of 3.0-5.0 g from the lower segment of the uterus (the decidua was cut off and disposed of); (II) strips of placenta of ca 5.0-10.0 g from the central part of its maternal side; (III) strips of fetal membranes of ca 3.0 g from the distal part. The strips were placed in hermetic test tubes after rinsing with 0.9% NaCl and stored for 3-6 weeks in -70°C; (IV) blood samples were obtained from antecubital veins without venous occlusion. The anticoagulant/blood proportion was 1:9 (one part of 3.2% sodium citrate, nine parts of the blood). The blood was placed in a plastic test-tube which was put in icy water and taken to the laboratory to be centrifuged (2500 x g, 20 min, +4°C). The blood plasma was divided into 200 µl portions in test tubes which were closed tightly and stored for 3-6 weeks at –70°C.

Preparation of the tissue extracts

To prepare tissue extracts we followed the procedure recommended by American Diagnostica Inc. In short, frozen tissue strips were pulverized within liquid nitrogen, then detergent extraction was applied (10% Triton X – 100 in Tris buffer, pH 8.5, 12 h at +4°C). The obtained suspended matter was centrifuged at 100 000 g for 60 min at +4°C. The supernatant was divided into 200 µl portions, placed in tightly closed plastic test-tubes, and kept for 1-2 weeks at -70°C.

Figure 1. **Concentration of cellular thrombomodulin (ng/mg of total protein) in placenta, fetal membranes and gestational myometrium, as well as soluble thrombomodulin in blood plasma of parturient and nonpregnant women**



Laboratory measurements

The concentration of thrombomodulin was measured by an immunoenzymatic method (ELISA). IMUBIND Thrombomodulin ELISA Kit by American Diagnostica GmbH was used. The manufacturer's instructions were strictly followed. The samples were assayed in batch operations. Total protein concentration was measured by BCA method using bicinchoninic acid. TM concentration was expressed in ng/mg of total protein. The interassay and intraassay coefficients of variability were less than 10%.

Statistical analysis

The results are presented as mean values with standard deviations (x±SD). Statistical analysis was done with Microsoft® Excel 2000 and Statistica for Windows 5.0 by StatSoft®. t-Student test was used for analysis of measurements in gestational tissues and mother's blood (dependent groups). Data between mother's blood and the blood of nonpregnant women (control) were analysed by unpared test of Mann-Whitney. Pearson's method was used for correlation analysis. The value $p<0.05$ was taken as statistically significant.

Results

1. Cellular TM in gestational tissues (placenta, fetal membranes and myometrium) (n=35):
Concentration of TM antigen in placenta was 18.756± ±3.826 ng/mg proteins, in fetal membranes 8.574±1.641 ng/mg proteins, and in the myometrium 4.721±1.935 ng/mg proteins. The differences between the particular values were highly significant ($p<0.0001$) (*Fig. 1*).

2. Soluble TM in mother's blood (n=35) and the blood of nonpregnant women (control) (n=20):

Concentration of TM antigen in the mother's blood plasma was 0.063±0.016 ng/mg proteins, and in blood plasma of nonpregnant women 0.098±0.040 ng/mg proteins. The difference was not significant statistically ($p>0.05$).

3. Comparison of TM concentration in blood and tissue extracts:

The ratios between soluble TM in blood plasma and cellular TM in tissue extracts were as follows: plasma/placenta – 1/298.4; plasma/fetal membranes – 1/136.5; plasma/myometrium – 1/74.6.

4. Correlation analysis:

No correlation was found between TM concentrations in plasma and placenta extract ($r=-0.1709$, $p=0.326$), blood plasma and fetal membranes extract ($r=-0.055$; $p=0.754$), as well as between concentrations in plasma and myometrium extract ($r=-0.0927$, $p=0.596$).

Discussion

A number of authors [2,17,18] have studied TM in placenta, but not yet in gestational myometrium and fetal membranes. In fact, we have found only one mention of TM measurements in 'uterus/ovary' of mice without information whether gestational myometrium was examined [19]. In our study we found out that TM is also present in gestational myometrium and fetal membranes, although in concentration lower than in the placenta. Originally TM was isolated from human placenta by Salem et al. in 1984 [17].

In placenta, TM was immunolocalized to syncytiotrophoblast and fetal vascular endothelium [2]. As concerns the localisation of TM in myometrium, we contemplate two options: either (I) TM comes from abundant vascularisation of gravid uterus, or/and (II) from the cytotrophoblast which invades spiral arteries of myometrium in the course of pregnancy [20]. We cannot exclude that TM measured by us in extract of fetal membranes might come from the fragments of decidua, which are broken off during labour. Anyway, immunohistochemical studies are needed to answer the question about localisation of TM in myometrium and fetal membranes.

Many hemostatic and nonhemostatic functions are ascriebed to placental TM.

TM is considered to be a major determinant of the antithrombogenity of placental bed via protein C anticoagulant pathway that controls the balance between coagulation and anticoagulation/fibrinolys [16,21]. As epithelial protein C receptors (EPCR) are also localized in syncytiotrophoblast [5], it would imply that TM activity in the intervillous space can be even amplified. Moniva [7] holds that APC is the main anticoagulant of intervillous space and shows that APC can release urokinase plasminogen activator (uPA) from uPA/PAI-1 complex enhancing in this way the protective fibrinolysis. In our working hypothesis we assume that myometrial TM can play a role similar to that of placental TM. The anatomic and functional integrity of uterine and placental segments of the utero-placental circulation might be a supplementary argument.

As regards to the clinical implications of our results, we assume that placenta and myometrium, when destroyed by retroplacental hematoma at placental abruption, can release TM into mother's blood and that would be the explanation for increased plasma level of TM in this complication which was observed by other authors [14].

Furthermore, cellular TM might be critical in reproduction, mainly in implantation and placentation as well as in early post-implantation embriogenesis. In animal experiments (mice), a deficit of TM or EPRC turned out to be a lethal factor [22]. Isermann et al. [23] have suggested that the thrombomodulin-protein C system is essential for maintaining pregnancy. Two distinct mechanisms were considered in intrauterine fetal growth retardation (IUGR): one of them is the result of engagement of protease-activated receptors (PAR's), PAR-2 and PAR-4 or both, by thrombomodulin-protein C system, and the second is TM-independent cytotoxic effect by fibrin degradation products on cytotrophoblast.

Taking into account and consideration our earlier studies on plasminogen activators and plasminogen activator inhibitors (PAs/PAIs system), and receptors for urokinase (uPAR) as well as tissue factor and tissue factor pathway inhibitor (TF/TFPI) in gestational myometrium [21,24,25], we would like to imply that gestational myometrium is parallely to placenta a source of many components that are active in coagulation and fibrinolysis. The presented results support the hypothesis that not only placenta but also myometrium can be the source of tissue TM, and that both, placental and myometrial TM can be of importance for intervillous blood fluidity.

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