Placenta 55 (2017) 21–28

Contents lists available at ScienceDirect

Placenta

journal homepage: www.elsevier.com/locate/placenta

Altered gene expression in human placenta after suspected preterm labour

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A R T I C L E I N F O

Article history:
Received 20 February 2017
Received in revised form
3 April 2017
Accepted 27 April 2017

Keywords:
Suspected preterm labour
Angiogenesis
Sterile inflammation
Fetal development
Placenta

A B S T R A C T

Introduction: Suspected preterm labour occurs in around 9% of pregnancies. However, almost two-thirds of women admitted for threatened preterm labour ultimately deliver at term and are considered risk-free for fetal development.
Methods: We examined placental and umbilical cord blood samples from preterm or term deliveries after threatened preterm labour as well as term deliveries without threatened preterm labour. We quantitatively analysed the mRNA expression of inflammatory markers (IL6, IFNγ, and TNFα) and modulators of angiogenesis (FGF2, PGF, VEGFA, VEGFB, and VEGFR1).
Results: A total of 132 deliveries were analysed. Preterm delivery and term delivery after suspected preterm labour groups showed similar increases in TNFα expression compared with the term delivery control group in umbilical cord blood samples. Placental samples from preterm and term deliveries after suspected preterm labour exhibited significantly increased expression of TNFα and IL6 and decreased expression of IFNγ. Suspected preterm labour was also associated with altered expression of angiogenic factors, although not all differences reached statistical significance.
Discussion: We found gene expression patterns indicative of inflammation in human placentas after suspected preterm labour regardless of whether the deliveries occurred preterm or at term. Similarly, a trend towards altered expression of angiogeneic factors was not limited to preterm birth. These findings suggest that the biological mechanisms underlying threatened preterm labour affect pregnancies independently of gestational age at birth.

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1. Introduction

Suspected preterm labour occurs in approximately 9% of all pregnancies [1].However, almost two-thirds of women admitted for suspected preterm labour do not deliver prematurely [2]. Women who deliver at term after suspected preterm labour are considered to have had an episode of “false preterm labour” that poses no risks to normal fetal development [3]. However, previous reports suggest that at-term infants whose mothers experienced an episode of suspected preterm labour could be at increased risk for fetal growth restriction [4–,8]. Randomized clinical trials assessing the effect of tocolysis suggest that after suspected preterm labour, early childhood cognitive outcomes are similar between at-term and late-preterm infants [9]. In line with these results, we recently reported an increased risk of suboptimal neurodevelopment in term-born 2-year-old children after an episode of suspected preterm labour [10]. Hence, in some cases, symptoms of

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http://dx.doi.org/10.1016/j.placenta.2017.04.025
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threatened preterm labour may result from a pathological insult whose nature or severity is not sufficient to induce irreversible spontaneous preterm delivery but that could alter the normal course of pregnancy because the fetus is subjected to an adverse intrauterine environment until delivery [4,11].

Preterm birth is the final common result of a variety of aetiologies with very different biological mechanisms [12], some of which are related to poor neonatal outcomes [13,14]. Previous efforts to prevent preterm birth have been hampered by a poor understanding of the underlying pathophysiology, inadequate diagnostic tools, and generally ineffective therapies [13]. Nevertheless, clinical, epidemiological, and experimental studies indicate that acute and chronic inflammatory conditions in the placenta is the most frequent underlying cause of preterm birth [15,16]. Alternatively, failed invasion by extravillous trophoblast cells leads to placental dysfunction, which results in adverse obstetric outcomes including pre-eclampsia, fetal growth restriction, and spontaneous preterm delivery. Various endothelial cell-derived angiogenic and antiangiogenic factors are important for the invasion process to proceed normally. These include vascular endothelial growth factor (VEGF), its receptor fms-like tyrosine kinase-1 (FLT1), soluble FLT1 (sFLT1), placental growth factor (PGF), and fibroblast growth factor 2 (FGF2) [17].

To determine how inflammatory and angiogenic pathways are affected by suspected preterm labour, we examined the expression patterns of genes with known inflammatory and angiogenic functions in samples of umbilical cord blood and placenta from preterm and term deliveries after suspected preterm labour.

2. Methods

2.1. Study population

We conducted a prospective cohort study of preterm (26–36 weeks) or term (≥37 weeks) delivery pregnancies after suspected preterm labour between September 2011 and May 2013 at the Hospital Clínico Universitario of Zaragoza, a tertiary university center. Suspected preterm labour was defined as the presence of regular and painful uterine contractions registered by cardiotocography and ultrasound cervical length <25 mm in the presence of intact membranes at gestational age of 24 + 0 to 36 + 6 weeks [18]. These groups were compared with a group of term delivery singleton pregnancies (≥37 weeks) without suspected preterm labour that were randomly sampled from our general obstetric population during the same time period. Last menstrual period was dated according to first-trimester crown-rump length [19]. Pregnancies involving congenital malformations, chromosomopathies, infections, and multiple gestations were excluded. The local ethics committee approved the study protocol (CEICA 14/2010), and parents provided written informed consent.

All pregnancies were managed by a staff obstetrician. Tocolysis with atosiban (Tractocile, Ferring Pharmaceuticals, Madrid, Spain), intramuscular betamethasone (2 × 12 mg/24 h), and magnesium sulphate was administered in some cases according to international clinical standards [20]. Maternal sociodemographic characteristics and clinical data were recorded in the hospital database at study inclusion. Based on the results of previous studies [21], the number of subjects required for detecting a >10% increase in markers of trophoblast inflammation assuming an alpha error of 5% and beta error of 20% was 110.

2.2. Placental and umbilical cord blood samples

Samples of the placenta and umbilical cord blood were collected immediately after delivery. We identified sites at the placental periphery of four lobules that were free of visible infarction, calcification, haematoma or damage, and four tissue samples (1–2 mm) were randomly removed from the parenchyma avoiding the maternal side of the placenta. Tissue samples were submerged in RNA Stabilization Solution (RNA*later*®, Fisher Scientific®), kept at 4 °C, and stored at −80 °C as suggested by the manufacturer.

2.3. Tissue homogenisation, RNA extraction, and mRNA expression analysis

Tissue stored at −80 °C in RNA*later*® was thawed on ice. Small pieces (200–400 mg) were cut from each specimen using dissection scissors and pooled tissue samples were mixed on ice with 1 ml ice-cold TRIzol™, and then 1.0-mm Zirconia beads (Biospec Products) were added. Tissue was disrupted on a Mini Beadbeater (Biospec Products) for 4 min at room temperature. Cell debris and other particulate matter was removed by centrifugation (3000 g) at 4 °C. The resulting homogenate was frozen at −80 °C until further use. RNA was subsequently extracted using an in-house method [22] based on standard TRIzol™ purification protocols (TRIzol®, Thermo Fischer Scientific) adapted from original protocols based on guanidinium thiocyanate [23]. After DNase I treatment as previously described [22], RNA was resuspended, its concentration was measured using Nanodrop, and RNA quality was determined in the Experion automated electrophoresis system (Bio-Rad Laboratories, Inc.) whose incorporated software automatically generated quality values expressed as RQ1. RNA was frozen at −80 °C until further use.

2.4. Reverse transcription (RT) and quantitative polymerase chain reaction (qPCR)

Total RNA was obtained from 104 high-quality samples, as clinical circumstances precluded taking samples from 11 patients, and 18 other samples were excluded due to inconsistencies in clinical records (n = 8) or inappropriate storage (n = 10). Only samples with RQ1 values > 3.3 were included in the study, although restricting the analysis to samples with RQ1 values > 6.0 did not change the outcome (data not shown). RNAs were retrotranscribed using the High-Capacity RNA-to-cDNA™ Kit (4387406, Applied Biosystems) at 20 ng RNA/μl reaction. TaqMan® assays (Life Technologies) were used to analyse the mRNA expression of the following markers (Hs numbers of assays indicated between barckets): IL6 (Hs00985639_m1), IFNγ (Hs00989291_m1), TNFα (Hs01113624_g1), FGF2 (Hs00266645_m1), PGF (Hs00182176_m1), VEGFA (Hs00900055_m1), VEGFB (Hs00173634_m1), and VEGFR1/FLT1 (Hs01052961_m1), using 18S rRNA (Hs03003631_g1) and GAPDH (Hs02758991_g1) or RPL19 (Hs02338565_gH) as reference genes. Reactions were carried out using TaqMan® Gene Expression Master Mix (4370074, Applied Biosystems) with 5 ng cDNA per reaction, oligos at 900 nM, and TaqMan probes at 250 nM in 10 μl reactions. All reactions were carried out in triplicate on a AB7900 HT apparatus (Applied Biosystems) using the following cycling protocol: 2 min at 50 °C, 10 min at 95 °C and 40 cycles of 15 s at 95 °C and 1 min at 60 °C. RT and qPCR assays were carried out by a commercial provider (Parque Científico de Madrid; www.fpcm.es).

Expression suite (ThermoFisher Scientific) was used to calculate ΔCt values with respect to the average of the Ct values of the 18S rRNA and GAPDH reference genes. No significant differences were observed using RPL19 [24] as a reference gene (data not shown). Data were further recalculated as 2-ΔΔCt, with respect to the mean ΔCt value of the samples in the control group as described [25]. The resulting numbers are listed in the Figures and represent fold change in expression.

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