Prostaglandins & other Lipid Mediators 123 (2016) 40–45

Contents lists available at ScienceDirect

Prostaglandins and Other Lipid Mediators

Original research article

Plasma C16-Cer levels are increased in patients with preterm labor

Piotr Laudanski[a,*], Karol Charkiewicz[a], Rafal Kisielewski[b], Pawel Kuc[a], Ewa Koc-Zorawska[c], Grzegorz Raba[d], Janusz Kraczkowski[e], Violetta Dymicka-Piekarska[f], Adrian Chabowski[g], Marian Kacerovsky[h], Bo Jacobsson[i,j], Piotr Zabielski[g], Agnieszka Blachnio-Zabielska[g]

[a] Department of Perinatology, Medical University of Bialystok, ul. Marii Sklodowskiej-Curie 24a, 15-276 Bialystok, Poland
[b] Department of Gynecologic Oncology, Oncology Center in Bialystok, ul. Ogrodowa 12, 15-057 Bialystok, Poland
[c] Department of Nephrology with Dialysis Centre, Medical University of Bialystok, ul. Zurawia 14, 15-540 Bialystok, Poland
[d] Institute of Obstetric and Emergency Medicine, University of Rzeszow, 37-710 Zurawica, Poland
[e] Department of Obstetrics and Pathology of Pregnancy, Medical University of Lublin, 20-081 Lublin, Poland
[f] Department of Clinical Laboratory Diagnostics, Medical University of Bialystok, J. Waszyngtona 15A, 15-269 Bialystok, Poland
[g] Department of Physiology, Medical University of Bialystok, ul. Mickiewicza 2C, 15-222 Bialystok, Poland
[h] Biomedical Research Center, University Hospital Hradec Kralove, Hradec Kralove, Czech Republic
[i] Department of Obstetrics and Gynecology, Institute for the Health of Women and Children, Sahlgrenska University Hospital, Göteborg, Sweden
[j] Department of Genes and Environment, Division of Epidemiology, Norwegian Institute of Public Health, Oslo, Norway

ARTICLE INFO

Article history:
Received 23 November 2015
Received in revised form 21 March 2016
Accepted 28 April 2016
Available online 13 May 2016

Keywords:
Preterm labor
Sphingolipids
C16 ceramide
Inflammation

ABSTRACT

Introduction: The pathogenesis of preterm labor is fragmentarily explained. The most widely accepted theory points out to infection and inflammation as possible causes, which can be mediated by potentially different factors, including sphingolipid mediators. Sphingolipids are a class of lipids that have been shown as important mediators in various cell processes such as: proliferation, growth, apoptosis, stress response, necrosis and inflammation. The aim of the study was to assess plasma concentrations of selected sphingolipids in patients with preterm labor.
Material and methods: We used ultra-high performance liquid chromatography with triple mass spectrometry (UHPLC-ESI–MS/MS) to assess plasma concentrations of the 11 sphingolipids in patients presenting with symptoms of preterm labor (n = 61) and threatened preterm labor (n = 40).
Results: We observed a statistically significant increase (p-value < 0.004) in plasma concentrations of C16-Cer in patients with preterm labor as compared to the control group. We also found C16-Cer to be the best predictor of preterm labor in the group of patients with symptoms occurring after 32 weeks of gestation.
Conclusions: Our findings show a possible involvement of selected sphingolipids, especially C16-Cer, in the pathogenesis of preterm labor. Their role as predictors of preterm delivery needs to be validated in the future on larger group of patients.

1. Introduction

Preterm labor is the leading cause of neonatal mortality throughout the world. Globally, an estimated 13 million babies are born preterm and approximately one million of them die annually. Prematurity is also associated with extremely high morbidity, as well as long-term physical impairment [1]. Preventing preterm labor remains a challenge because the causes of preterm births in many cases remain unknown, despite large-scale scientific efforts in this field of medicine [2–6].

In women presenting at the hospital with symptoms of preterm labor, only few will actually deliver preterm. Taking into account the possible harmful effects of tocolytic and steroid therapies, as well as high costs of medical care, it is important to early identify which women with threatening preterm delivery will actually have preterm birth [7]. Currently, cervical length, uterine activity monitoring and various biochemical markers (fetal fibronectin, salivary estriol or insulin-like growth factor binding protein-1) are used to predict preterm delivery [8]. However, none of them is specific enough. Among many theories attempting to explain the pathogenesis of preterm uterine contractions and preterm prelabor rupture

* Corresponding author.
E-mail address: plauda@umb.edu.pl (P. Laudanski).

http://dx.doi.org/10.1016/j.prostaglandins.2016.04.005
1098-8823/

of membranes (pPROM), subclinical inflammation seems to play the pivotal role [9,10]. Such view is supported by microbiological studies showing that up to 40% of preterm deliveries result from infection, which can be reflected by typical histological features in the placenta, umbilical cord and amniotic fluid, as well as many biochemical markers of inflammation [11,12].

Although research has demonstrated potential roles for disruptions in endocrine, immune, and other biochemical pathways in precipitating preterm birth, the precise pathogenesis remains unknown. There are several factors known in pathology of preterm birth but not in normal gestation. The key mediators of inflammatory response in preterm labor are IL-1 beta and TNF-alpha, which enhance prostaglandin production by inducing COX-2 expression in decidua and amnion. In the chorion the prostaglandin metabolizing enzyme (15-hydroxyprostaglandin dehydrogenase —PGDH) is inhibited. Prostaglandins play a central role in the initiation of labor by inducing myometrial contractility, cervix ripening and membrane rupture [13,14]. We showed in our previous work the decreased serum level of macrophage inflammatory chemokine-3beta/CCL19 in preterm labor as compared to delivery at term [15,16]. In other work we found that the concentration of 3 chemokines, that is, I-TAC, MIP-3α, and TARC above the cut-off value and HCC-4 below the cut-off value, indicate the risk of preterm delivery [17]. Ugur et al. reported increased sialic acid levels of the preterm labor patients as compared with term deliveries [18]. Numerous other studies shows possible implication of other inflammatory cytokines, mainly Il-6 and CRP in pathogenesis of preterm delivery [19]. It is likely that inflammatory cytokines as well as sphingolipids bring about parturition through increasing prostaglandin levels by manipulating prostaglandin production. The elucidation of how these cytokines mediate such effects and whether their effects can be changed by treatments merit further research.

Sphingolipids are a group of lipids containing organic aliphatic amino alcohol sphingosine backbone or a substance structurally similar to it (e.g. sphinganine or phytosphingosine). They have long been thought to be structural components of the outer leaflet of the plasma membrane lipid bilayer. Many studies have focused on the participation of these compounds in various cell processes such as: proliferation, growth, differentiation, apoptosis, stress response, and senescence [20]. There are also numerous data showing implication of sphingolipids in various pathways associated with inflammation and immunity [21–23]. There are limited data documenting the involvement of sphingolipid mediators in the pathogenesis of labor [24–28]. Taking into account the importance of sphingolipids in inflammatory processes, and possibly in the pathogenesis of preterm birth, the main aim of this study is to assess plasma concentrations of selected sphingolipids in patients presenting with threating preterm delivery (false and true preterm labor).

2. Material and methods

The study group consisted of patients who delivered at tertiary centers as it was described in our recent open-access paper [29].

Patients with clinical chorioamnionitis, i.e. white blood cell count >18.000, temperature elevation of >37.8 °C, tachycardia, uterine tenderness greater than expected, and foul-smelling vaginal discharge were excluded from the study. CRP levels in every case were within the normal values (no more than 10 mg/l).

We also excluded patients who received antibiotics and corticosteroids at least three days before blood sampling as well as patients with: multiple pregnancy, pregnancy induced hypertension, diabetes, kidney disease (creatinine above 2 mg/dl) and other complications during pregnancy, such as anemia, thrombocytopenia, systemic disease, and taking into consideration that lipids play an important role in the pathogenesis of metabolic syndrome, exclusion criteria comprised pregestational body mass index (BMI) of >35 and vascular diseases.

Written informed consent was obtained from all women enrolled in the study, after clear verbal and written explanation about the goal and methodology of the investigation. The study protocol was approved by the Local Ethical Committee of Medical University of Bialystok, Poland, and informed consent was obtained from each patient.

10 ml of blood samples and 10 ml of urine were obtained from patients presenting with symptoms of threatening preterm labor such as: ruptured fetal membranes, regular painful uterine contractions (at least two contractions every 10 min), accompanied by cervical change (dilation and/or effacement). The diagnosis of preterm labor (group I) was made on the basis of previously established criteria [15,30]. In all these patients, labor started with regular contractions and progressive cervical dilation (n = 27) or preterm premature rupture of membranes (pPROM) – (n = 34). The diagnosis of pPROM was defined as the presence of vaginal pooling with positive Amnisure test (Qiagen) prior to regular uterine activity.

There are sub-categories of preterm birth, based on gestational age:

- extremely preterm (<28 weeks)
- very preterm (28 to <32 weeks)
- moderate to late preterm (32 to <37 weeks).

This division was initially made for neonatal care. Infants born below 28 weeks gestation have extreme morbidity and mortality rates. On the other hand children delivered in the late preterm period will generally not suffer any serious complications [31].

Available data shows that this division is also important in perinatology. Each of this period differs in relation to pathophysiology. For example, the majority of extremely preterm births is the result of infection [31].

Blood and urine samples were subsequently centrifuged, plasma and urine separated and frozen at −80 °C. The collected material was then transferred to the Department of Physiology, Medical University of Bialystok, Poland.

Ultra high-performance liquid chromatography (UHPLC) (Agilent1290) and electrospray ionization triple mass spectrometry (Agilent6460) (UHPLC-ESI–MS/MS) was used to determine plasma and urine concentrations of the selected sphingolipids according to Blachnio-Zabielska et al. [32,33]. Briefly, the plasma samples were spiked with internal standard stock solution (d17:1-Sph, d17:1-S1P and C17:0-Cer in ethanol) and subsequently extracted with isopropanol/water/ethyl acetate (35:5:60, v/v/v). Extracts were evaporated to dryness under a stream of nitrogen and suspended in buffer A (methanol, 2 mM ammonium formate, 0.1% formic acid). Samples were eluted from C18 UHPLC column with buffer B (water, 1 mM ammonium formate, 0.1% formic acid) at the flow rate of 0.4 ml/min. The following sphingolipids were measured against standard curves prepared on commercially available standards (Avanti Polar Lipids): Sphingosine (Sph), Sphinganine (SPA), Sphingosine-1-Phosphate (S1P), C14 Ceramide (C14-Cer), C16 Ceramide (C16-Cer), C18:1 Ceramide (C18:1-Cer), C18 Ceramide (C18-Cer), C22 Ceramide (C22-Cer), C24:1 Ceramide (C24:1-Cer), C24 Ceramide (C24-Cer). This technique in analytic chemistry is fast, highly specific and sensitive in separating, identifying and quantifying components in the mixture. As it has recently become apparent, it is also the method of choice in sphingolipidomics [34,35].

In order to assess the concentration of tumor necrosis factor alpha (TNF-α) and prostaglandin E2, we used Human TNF-alpha