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Placental dimethyl acetal fatty acid derivatives are elevated in preeclampsia

M. Brien [a], L. Berthiaume [a], I. Rudkowska [a, b], P. Julien [a, c], J.F. Bilodeau [a, c, *]

[a] Axe endocrinologie et néphrologie, Centre de recherche du CHU de Québec-Université Laval, et Centre de recherche en endocrinologie, métabolisme et inflammation (CREMI), Université Laval, Québec G1V 4G2, Canada
[b] Département de Kinésiologie, Faculté de médecine, Université Laval, Québec G1K 7P4, Canada
[c] Département de médecine, Faculté de médecine, Université Laval, Québec G1K 7P4, Canada

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ABSTRACT

Preeclampsia (PE) was shown to affect the placental content and the transfer of polyunsaturated fatty acids (PUFA) to the fetus. Plasmalogens, a type of phospholipids with a vinyl-ether link at the sn-1 position, play an antioxidant role and are specifically enriched in PUFA at the sn-2 position. In this study, we characterized plasmalogen-derived dimethyl acetal (DMA) fatty acid derivatives, 16:0 DMA, 18:0 DMA, 9*c*-/11*c*-18:1 DMA and PUFA in the placenta of normotensive (n = 20) and PE (n = 20) pregnancies, according to the sampling site: peri-insertion or periphery. Phospholipid fatty acids from the placenta and maternal erythrocytes were identified by gas chromatography mass spectrometry and quantified by flame ionization detection. We found elevated total DMA in the PE placenta by 18% when compared to normotensive controls ($p = 0.026$). Moreover, the 16:0 DMA account for more than 55% of DMA fatty acids measured in the placenta, and its level is significantly higher in PE than controls ($p = 0.018$). Also, we found elevated placental PUFA, 20:5(n-3), 22:5(n-3) and a low level of 20:4(n-3) in PE compared to controls. Placental DMA was highly correlated with n-6 and n-3 PUFA in both, normotensive and PE pregnancies. In sum, elevated DMA fatty acids in the PE placenta could be an indirect defensive mechanism against oxidative stress and poor placental fatty acid transfer in PE.

1. Introduction

Preeclampsia (PE) is a common and complex pregnancy syndrome characterized by the appearance of hypertension and proteinuria after 20 weeks of gestation [1]. Various etiologies could be involved in PE but it is well-established that an abnormal placental development is an essential and early step in the pathophysiology [2]. Insufficient spiral arteries remodeling in PE was associated to placental oxidative stress and the generation of oxidized fatty acids (reviewed in Ref. [3]).

The placental abnormalities in PE were also shown to affect negatively the transport and the synthesis of fatty acids, especially omega-3 (n-3) polyunsaturated fatty acids (PUFA) like docosahexaenoic acid (DHA) [4]. Omega-6 (n-6) and n-3 PUFA can either be provided by the diet or synthesized by their shorter chain precursors, linoleic (LA) and α-linolenic (ALA) acids respectively. Maternal supplementation with n-3 PUFA from fish oil or fortified milk showed beneficial effects in terms of gestation length [5], birth weight [6], decreased placental apoptosis [7] and lowering the risk of preterm birth or PE [8].

The phospholipids are a reservoir of fatty acids and key components of lipoproteins and cellular membranes of erythrocytes and placenta. Plasmalogens are a special type of phospholipids characterized by a vinyl-ether link at the sn-1 position [9]. The presence of this link enable plasmalogens to act as an endogenous antioxidant to prevent lipid oxidation [10–12]. The acid-catalyzed hydrolysis of the vinyl ether bond of plasmalogens yield to the generation of dimethyl acetal (DMA) fatty acid derivatives, an

Abbreviations: 9*c*-18:1, oleic acid; 11c-18:1, vaccenic acid; 16:0, stearic acid; AA, arachidonic acid / 20:4(n-6); AdA, adrenic acid / 22:4(n-6); ALA, α-linolenic acid / 18:3(n-3); DGLA, dihomo-γ-linolenic acid / 20:3(n-6); DHA, docosahexaenoic acid / 22:6(n-3); DMA, dimethyl acetal; DPA, docosapentaenoic acid / 22:5(n-6); EPA, eicosapentaenoic acid / 20:5(n-3); ETA, eicosatetraenoic acid / 20:4(n-3); GLA, γ-linolenic acid / 18:3(n-6); iTFA, industrial trans fatty acids (e.g. 9*t*-18:1); LA, linoleic acid / 18:2(n-6); MUFA, monounsaturated fatty acids; n-3 DPA, 22-5(n-3); PE, preeclampsia; PUFA, polyunsaturated fatty acids; rTFA, ruminant trans fatty acids (e.g. 11*t*-18:1); SDA, stearidonic acid / 18:4(n-3); SFA, saturated fatty acids.

* Corresponding author. Centre de recherche du CHU de Québec, Axe endocrinologie et néphrologie, 2705 boulevard Laurier, Québec (Qc) G1V 4G2, Canada.
E-mail address: jean-francois.bilodeau@crchudequebec.ulaval.ca (J.F. Bilodeau).

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Table 1
Clinical characteristics of the normotensive and preeclamptic groups.

	Normotensive ($n = 20$)	Preeclampsia ($n = 20$)	p
Maternal age (years)	29.0 ± 0.7	30.0 ± 1.5	0.960
Gestational age at sampling (weeks)	40.5 ± 0.2	36.4 ± 0.7*	<0.001
Pre-pregnancy BMI (m^2/Kg)	22.5 ± 1.0	26.7 ± 1.9	0.135
Mean systolic blood pressure (mmHg)	119 ± 2	161 ± 4*	<0.001
Mean diastolic blood pressure (mmHg)	76 ± 2	99 ± 3*	<0.001
Urinary protein excretion (g/24 h)	<0.3	1.8 ± 0.6*	<0.001
Vaginal delivery	19	15	
Caesarian section	1	5	
Smokers	1	4	

Values are means ± SEM.
BMI: body mass index.
*Values are statistically different from control, Mann Whitney test, $p < 0.05$.

indirect indicator of plasmalogens [13].

Plasmalogens are formed endogenously by the peroxisomes and can also be provided by the diet. Indeed, anaerobic bacteria implicated in lipid metabolism of the ruminants are enriched in plasmalogens and thus influence the lipid composition of ruminant tissues and milk [14]. Plasmalogens are also preferentially enriched in PUFA at the sn-2 position (reviewed in Ref. [9]). Furthermore, plasmalogens are known to be modulated in PE since 18:0 DMA and 18:1 DMA levels were reported to be significantly decreased in erythrocytes compared to control pregnancies [15].

The aim of this study was to compare placental plasmalogen FA profiles of normotensive and PE pregnancies. Since placenta is a heterogeneous organ, we assessed the regional variations of FA near the umbilical cord and the periphery [16]. We used erythrocyte membranes FA composition from maternal blood to estimate the dietary intake of FA. The erythrocytes were shown to reflect the longer-term intake of FA from food sources over approximately 120 days [17]. We are the first to investigate placental levels of DMA fatty acid derivatives in PE.

2. Materials and methods

2.1. Patients recruitment

Pregnant women were recruited at the Centre mère-enfant of Centre hospitalier universitaire de Québec – Université Laval. The study was approved by the local ethic committee and all subjects signed an information-consent form. PE women were recruited at admission according to a systolic and diastolic blood pressure of respectively, 140 and 90 mm Hg or higher, on two separate readings at least 4 h accompanied with proteinuria ≥0.3 g/day (+1 or greater on dipsticks) in accordance with the Canadian Hypertension Society Consensus [18]. Normotensive pregnant women were characterized by a normal blood pressure (≤140/90 mm Hg), and absence of urine protein excretion and medical complications. Pregnant women presenting any pre-existing medical conditions (chronic hypertension, diabetes mellitus, kidney disease, blood-clotting disorders) were excluded. Other exclusions factors were extreme age (<18 or >40 years old) and the intake of anticoagulant drugs or drugs affecting lipid metabolism.

2.2. Blood collection and processing

Blood samples from normotensive and PE pregnancies were collected in heparinized tubes 24 h before delivery. Plasma was separated from erythrocytes and other blood cells by centrifugation (10 min, 180×*g* at room temperature) and each fraction was frozen at −80 °C. Just before determination of fatty acids, the frozen pellet of blood cells was washed twice with 0.9% NaCl solution and centrifuge (21,000×*g*, 15 min) to isolate membranes of lysed erythrocytes.

2.3. Placental sample collection and processing

The sampling procedure was previously described [19]. Briefly, we manually sampled villi from each placenta at the peri-insertion (within 2.5 cm from the insertion of the umbilical cord), and periphery (2.5 cm from the placental margin). Immediately after sampling, tissues were washed in a PBS 1X solution and then snap frozen at −80 °C until analyzed.

Just before the analysis of fatty acids, placental villi samples of 0.3 g were homogenized with 1150 μl of water, 50 μl of 0.1% BHT solution and 50 μl of indomethacin 625 μM solution using a tissue homogenizer (VWR international, Ville Mont-Royal, Qc, Canada). Tubes were weighted in order to determine the tissue concentration of the homogenates.

2.4. Fatty acid analyses

A solution of chloroform: methanol (2:1 v/v) was used to extract lipids from erythrocytes membranes and placental homogenates as previously described [20,21]. Briefly, the isolated phospholipid fatty acids were methylated following a transesterification reaction using a mix of methanol:benzene (4:1) and acetyl chloride at 95 °C for 1.5 h. Fatty acids methyl esters (FAME) and DMA derivatives were identified by gas chromatography (GC) coupled mass spectrometry (MS) and quantified by GC coupled to flame ionization detector (FID) as explained elsewhere [22]. The GC model 7890 coupled to a MS model 5977B were from Agilent Technologies (Mississauga, ON, Canada). The capillary column used was a HP-88 (100 m × 0.25 mm, 0.20 μm, Agilent technologies) and the carrier gas was helium at 1.5 ml/min [22]. Standard for C16:0 DMA (1,1-dimethoxyhexadecane), C18:0 DMA (1,1-dimethoxyoctadecane) and C18:1 DMA (9Z-octadecenal-dimethylacetal) were purchased at Avanti Polar Lipids (Alabaster, Alabama, USA).

2.5. Statistical analyses

Statistical analyses were performed with GraphPad Prism 7.0 (GraphPad Software Inc., 2014, San Diego, USA). The Mann Whitney test was used to compare levels of fatty acids in erythrocytes since normal distribution was not reached using d'Agostino & Person omnibus test. Placental fatty acids values were transformed to the natural logarithm to meet the assumptions of normality and homogeneity of variance. Then, a two-way analysis of variance with repeated measures was used to study the effects on fatty acids levels of the syndrome (control, PE) and the sampling site (peri-insertion, periphery) of the placenta. For all these analyses, a p-