Placenta 34 (2013) 1095–1098

Contents lists available at ScienceDirect

Placenta

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

Short communication

A parallel increase in placental oxidative stress and antioxidant defenses occurs in pre-gestational type 1 but not gestational diabetes

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ARTICLE INFO

Article history:
Accepted 2 September 2013

Keywords:
Maternal diabetes
Oxidative stress
Placenta

ABSTRACT

We aimed to determine the oxidative stress status in placentas obtained from gestational (GDM) and type 1 (T1D) diabetic pregnancies. Malonaldehyde and protein carbonyls, two biomarkers of oxidative damage, were higher in T1D but not in GDM placentas. Also, higher reduced glutathione and lower oxidized glutathione levels and higher glutathione peroxidase activity were found in T1D but not in GDM placentas. These results suggest that T1D placentas may develop a protective antioxidant mechanism to overcome higher oxidative stress levels.

1. Introduction

Diabetes is the most prevalent metabolic disorder diagnosed in pregnant women [1,2]. Both gestational (GDM) and pre-gestational type 1 diabetes (T1D) are associated with an increased risk of adverse perinatal outcomes and later in life metabolic diseases for both the mother and the offspring [2,3]. While maternal hyperglycemia is recognized as the main factor responsible for those complications [3,4], other factors, such as oxidative stress, also seem to play an important role in the pathophysiology of maternal diabetes and its complications [3]. Increased levels of oxidative stress have been found in diabetic pregnant women. However, the majority of these studies analyzed maternal tissues such as plasma and serum [5–7], and only a few analyzed both GDM and T1D human placentas [8].

So, in order to test the hypothesis that diabetic pregnancy is associated with an increase in placental oxidative stress, we decided to compare the placental oxidative stress status in uncomplicated, GDM and T1D pregnancies.

2. Materials and methods

Following ethical approval, human placentas were collected at the Department of Obstetrics and Gynecology of Centro Hospitalar S. João, Porto, from uncomplicated (control), GDM and T1D singleton term pregnancies (Table 1), within half an hour after spontaneous delivery or elective cesarean section. GDM was diagnosed according to published criteria defined by the International Association of the Diabetes and Pregnancy Study Group (IADPSG) consensus panel [9]. None of these pregnancies were associated with any major maternal or fetal pathology or diabetes-associated complications.

Placental villous tissue homogenates were incubated in the absence or presence of *tert*-butyl hydroperoxide (*tert*-BOOH) 3 mM for 1 h at 37 °C, as previously described [10]. Afterwards, levels of malonaldehyde (MDA), protein carbonyls, total (GSX), oxidized (GSSG) and reduced (GSH) glutathione and the activity of Se-dependent glutathione peroxidase (GPX) were quantified in supernatants as described elsewhere [10,11].

Statistical significance ($P < 0.05$) between various groups and two groups were analyzed by one-way ANOVA (followed by the Bonferroni test) and Student's *t* test, respectively. Pearson correlation coefficients were calculated to determine significant associations between maternal metabolic parameters and oxidative stress.

3. Results and discussion

Biomarkers of oxidative damage to lipids (MDA) and proteins (carbonyls) [12] were elevated in T1D, but not in GDM placentas, when compared to control (Fig. 1a and b), indicating that greater lipid and protein oxidation levels exists in T1D placentas only. Exposure to an oxidative challenge (*tert*-BOOH) induced an increase in MDA levels in all groups, although the relative increase was smaller in T1D (2×) than in control and GDM placentas (9 and 11×, respectively) (Fig. 1a). By contrast, protein carbonyl levels were not changed by *tert*-BOOH (Fig. 1b).

Hyperglycemia is one of the most important mechanisms leading to increased oxidative stress in GDM [1,5,13]. However, no correlations were found between placental biomarkers of oxidative stress and maternal third trimester fasting glycemia or glycosylated

Abbreviations: GDM, gestational diabetic pregnancies; GSX, total glutathione; MDA, malonaldehyde; T1D, pre-gestational type 1 diabetic pregnancies; *tert*-BOOH, *tert*-butyl hydroperoxide.

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http://dx.doi.org/10.1016/j.placenta.2013.09.001

Table 1
Clinical characteristics of the three study groups.

	Control	GDM[a]	T1D[b]
Mothers			
N	11	15	5
Maternal age (years)	33.1 ± 1.3	34.9 ± 0.9	27.2 ± 2.9*#
BMI before delivery (Kg/m^2)[c]	26.5 ± 1.6	31.6 ± 1.0*	23.7 ± 1.4#
Gravida (*n*)	2.4 ± 0.4	2.3 ± 0.2	1.8 ± 0.6
Parity (*n*)	0.9 ± 0.3	1.3 ± 0.2	0.6 ± 0.4
Mode of delivery			
Vaginal [*n* (%)]	4 (36)	6 (40)	2 (40)
Cesarean [*n* (%)][d]	7 (64)	9 (60)	3 (60)
Therapeutics of GDM [*n* (%)]	–	No insulin: 8 (53) Insulin[e]: 7 (47)	–
Fasting blood glucose (mM)[f]			
All	3.9 ± 0.1	4.8 ± 0.2*	7.9 ± 1.6*#
Insulin therapy		4.4 ± 0.3	
No insulin therapy		5.0 ± 0.2*	
HbA_{1C} (%)[g]			
All	–	5.6 ± 0.1	6.3 ± 0.4
Insulin therapy		5.6 ± 0.2	
No insulin therapy		5.7 ± 0.2	
Periconceptional FA use [*n* (%)][h]	10 (90)[i]	14 (93)[i]	3 (60)[i]
Smokers [*n* (%)][j]	0 (0)	0 (0)	1 (20%)
Infants			
Gestational age at birth (weeks)[k]	39.6 ± 0.3	39.2 ± 0.2	37.6 ± 0.7*#
Birth weight (g)[l]	3200 ± 128	3486 ± 138	3293 ± 166
Length (cm)[m]	48.2 ± 0.5	49.6 ± 0.4*	47.9 ± 1.0
SGA newborn [*n* (%)][n]	1 (9)	0 (0)	0 (0)
AGA newborn [*n* (%)]	9 (82)	11 (73)	5 (100)
LGA newborn [*n* (%)]	1 (7)	4 (29)	0 (0)
Placental weight (g)	621.7 ± 49.6	702.3 ± 41.9	588.0 ± 48.9
Sex [*n* (%)]	Male: 2 (18) Female: 9 (82)	Male: 7 (47) Female: 8 (53)	Male: 3 (60) Female: 2 (40)
5-min Apgar Score	9.5 ± 0.2	9.3 ± 0.2	10.0 ± 0.0#

Values represent mean ± SEM.
*Significantly different from control ($P < 0.05$); #significantly different from GDM ($P < 0.05$).
[a] GDM, gestational diabetic pregnancies.
[b] T1D, pre-gestational type 1 diabetic pregnancies.
[c] BMI, body mass index.
[d] All cesarean sections were elective except one from control and one from GDM group, which were laboring.
[e] The criteria for initiating insulin therapy was the presence of a fasting glycemia ≥90 mg/dL (5 mM) or a 2 h-postprandial blood glucose level ≥120 mg/dL (6.7 mM), despite consistent dietary and exercise adjustments.
[f] The majority of values were obtained at 24–28 weeks of gestation. Parameter unknown for 3 subjects from GDM group and 1 from T1D group. Women with T1D were treated with intermediate or long-acting insulin regimens in order to achieve a fasting glycemia <90 mg/dL and a 2 h-postprandial blood glucose level <120 mg/dL.
[g] Values obtained at 35–36 weeks of gestation. Parameter unknown for one subject from GDM group and for all subjects from control group, as this assay is not typically ordered for subjects with no history of glucose mismanagement.
[h] FA, folic acid. Dosage and initiation period unknown.
[i] Parameter unknown for 1 subject.
[j] Parameter unknown for 2 subjects from control group and 1 from T1D group.
[k] Gestational age: number of completed weeks at the time of delivery, determined by prenatal ultrasound at 11–13 weeks.
[l] Birth weight was evaluated to the nearest gram.
[m] Length was evaluated to the nearest tenth of a centimeter after birth.
[n] SGA, small-for-gestational-age; AGA, adequate-for-gestational-age; LGA, large-for-gestational-age, classified according published reference standards (Battaglia FC, Lubchenco LO (1967) A practical classification of newborn infants by weight and gestational age. J Pediatr 71:159–163).

hemoglobin (HbA_{1C}) in GDM pregnancies (which do not present overt hyperglycemia at this time point) (Table 1). Also, no correlations were observed between biomarkers of oxidative stress, body mass index and gestational or maternal age in GDM women (results not shown). Due to the low number of T1D placentas analyzed ($n = 5$), correlation data are not shown.

Glutathione and GPX are reliable indicators of antioxidant status [13]. When compared to control and GDM placentas, higher GSH and lower GSSG concentrations (Fig. 2b and c) and a higher [GSH]/[GSSG] ratio (78 ± 45 for T1D, 1.3 ± 0.4 for GDM, and 0.8 ± 0.3 for

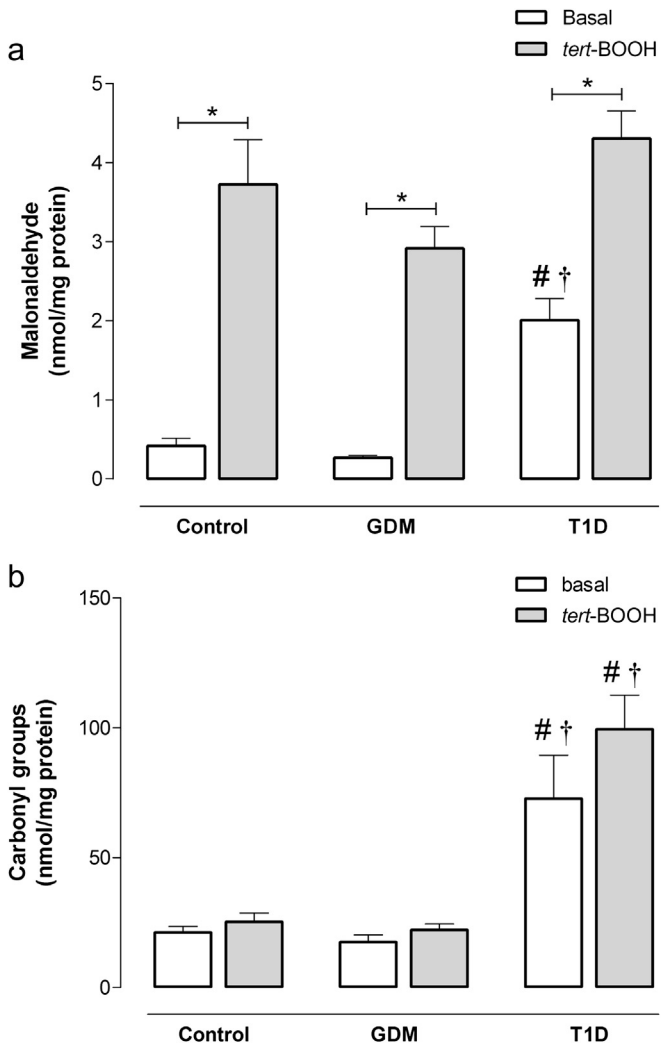

Fig. 1. Levels of malonaldehyde (a) and protein carbonyls (b) in placentas from control ($n = 11$), gestational diabetic (GDM; $n = 15$) and pre-gestational type 1 diabetic (T1D; $n = 5$) pregnancies. These parameters were determined in human placental homogenates after a 1 h-exposure to 3 mM *tert*-butyl hydroperoxide (*tert*-BOOH) or its solvent (basal). Shown are arithmetic means + S.E.M. #Significantly different from control placentas with the same treatment ($P < 0.05$). †Significantly different from GDM placentas with the same treatment ($P < 0.05$). *Significantly different from basal ($P < 0.05$).

control) were found in T1D placentas. On the other hand, GSX, GSH and GSSG levels and [GSH]/[GSSG] ratios were similar in GDM and control placentas (Fig. 2a–c). In the presence of *tert*-BOOH, the decrease in GSH levels was greater in T1D than in control and GDM placentas, suggesting that T1D glutathione is more sensitive to an additional oxidative challenge (Fig. 2b). Additionally, GPX basal activity was higher in T1D but not in GDM placentas in comparison to control, but exposure to *tert*-BOOH decreased the enzyme activity to similar levels in all groups (Fig. 2d).

As a whole, these results suggest that a compensatory antioxidant mechanism – upregulation of glutathione/GPX system – may develop in T1D placentas to overcome higher oxidative stress levels. In agreement with our results, a parallel increase in blood levels of oxidative stress biomarkers and antioxidant enzymes activity have been reported in T1D pregnant women [14,15].

Concerning GDM, we did not find any effect on placental oxidative stress and antioxidants levels, even after stratification for insulin and non-insulin therapy and for labored (vaginal) or non-labored (elective cesarean section) deliveries (results not shown). By contrast, in controls, levels of MDA were more sensitive to *tert*-