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Placental antioxidant enzyme status and lipid peroxidation in pregnant women with type 1 diabetes: The effect of vitamin C and E supplementation

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ABSTRACT

Aim: In view of the increased rates of pre-eclampsia observed in diabetic pregnancy and the lack of ex vivo data on placental biomarkers of oxidative stress in T1 diabetic pregnancy, the aim of the current investigation was to examine placental antioxidant enzyme status and lipid peroxidation in pregnant women with type 1 diabetes. A further objective of the study was to investigate the putative impact of vitamin C and E supplementation on antioxidant enzyme activity and lipid peroxidation in type 1 diabetic placentae.
Methods: The current study measured levels of antioxidant enzyme [glutathione peroxidase (Gpx), glutathione reductase (Gred), superoxide dismutase (SOD) and catalase] activity and degree of lipid peroxidation (aqueous phase hydroperoxides and 8-iso-prostaglandin F2α) in matched central and peripheral samples from placentae of DAPIT (n = 57) participants. Levels of vitamin C and E were assessed in placentae and cord blood.
Results: Peripheral placentae demonstrated significant increases in Gpx and Gred activities in pre-eclamptic in comparison to non-pre-eclamptic women. Vitamin C and E supplementation had no significant effect on cord blood or placental levels of these vitamins, nor on placental antioxidant enzyme activity or degree of lipid peroxidation in comparison to placebo-supplementation.
Conclusion: The finding that maternal supplementation with vitamin C/E does not augment cord or placental levels of these vitamins is likely to explain the lack of effect of such supplementation on placental indices including antioxidant enzymes or markers of lipid peroxidation.

1. Introduction

Pre-eclampsia is defined by the new onset of hypertension during pregnancy accompanied by the development of proteinuria, it is associated with significant maternal and perinatal morbidity and mortality. Although our understanding of the pathogenesis of pre-eclampsia has improved significantly, the exact aetiology is still not fully understood, however the pathophysiology involves abnormal placental development during the first trimester with resulting placental insufficiency and the subsequent release of placental factors into maternal circulation resulting in the clinical syndrome of pre-eclampsia (Lyall, Bulmer, Duffie, Cousins, et al., 2001; Redman & Sargent, 2005). Oxidative stress plays an important role in the normal inflammatory response of pregnancy, however, evidence has been provided that pregnancies complicated by pre-eclampsia are associated with increased concentrations of oxidative stress markers including lipid peroxidation products, and a reduction in antioxidant concentrations (Chappell, Seed, Briley, Kelly, et al., 2002; Hubel, Mc Laughlin, Evans, Hauth, et al., 1996). Most studies have examined systemic markers of oxidative stress and antioxidant status, limited analyses have suggested that placental oxidative stress is also present in pre-eclampsia (Gupta, Aziz, Sekhon, Agarwal, et al., 2009). The antioxidant enzymes superoxide dismutase (SOD), glutathione peroxidase (Gpx), glutathione reductase (Gred) and catalase are essential in scavenging hydrogen peroxide which potentiates injury at the cellular level (Krinsky, 1992). Numerous studies have examined the relationship between exogenous antioxidant supplementation and their impact on reducing pre-eclampsia risk, however these studies were derived mainly from non-diabetic populations (Beazley, Ahokas, Livingston, Griggs, et al., 2005; Chappell, Seed, Briley, Kelly, et al., 1999; Klemmensen, Tabor, Østerdal, Knudsen,

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et al., 2009; Rumbold, Crowther, Haslam, Dekker, et al., 2006; Villar, Purwar, Merialdi, Zavaleta, et al., 2009).

Rates of pre-eclampsia are 2–4 times higher in diabetic pregnancy in comparison to their non-diabetic counterparts, furthermore, pregnancy outcomes in the UK remain worse for women with pre-existing diabetes (Person, Norman, & Hanson, 2009). The Diabetes and Pre-Eclampsia Intervention Trial (DAPIT) addressed the impact of vitamin C/E supplementation on the risk of pre-eclampsia in a large population of type 1 diabetic women (McCance, Holmes, Maresh, Patterson, et al., 2010). In DAPIT the authors also demonstrated that in women who developed pre-eclampsia, glycaemic control was significantly higher before and during pregnancy compared with women who did not develop pre-eclampsia (Holmes, Young, Patterson, Pearson, et al., 2011). The DAPIT study also showed that poor glycaemic control was associated with the development of pre-eclampsia but not pregnancy induced hypertension. These data are important in terms of their support for putative relevance of poor glycaemic control to increased production of free radical species (lipid peroxidation) with inadequate antioxidant defence mechanisms in the pathogenesis of pre-eclampsia.

A systematic review and meta-analysis of nine RCTs of supplementation with vitamins C/E for the prevention of pre-eclampsia has concluded that such supplementation does not prevent pre-eclampsia (Agudelo, Romero, Kusanovic, & Hassan, 2011). There is a lack of ex vivo data on placental biomarkers of oxidative stress in T1 diabetic pregnancy and limited evidence regarding the impact of antioxidant vitamin supplementation on placental indices in pre-eclamptic women. Therefore, the aim of the current study using placentae from a DAPIT sub-cohort was to investigate placental antioxidant enzyme (Gpx, Gred, SOD and catalase) activity and the degree of lipid peroxidation, as measured by aqueous phase hydroperoxides and 8-iso-prostaglandin F2α, in non-pre-eclamptic pregnancies and those complicated by pre-eclampsia. A further aim of the study was to investigate the putative impact of vitamin C and E supplementation on antioxidant enzyme activity and lipid peroxidation in type 1 diabetic placentae.

2. Materials and methods

2.1. Subjects

Pre-eclampsia was defined as gestational hypertension with proteinuria using the International Society for the Study of Hypertension in Pregnancy guidelines (Davey & Mac Gillivray, 1998). Each case of hypertensive pregnancy was reviewed by the staff of the Trial Coordinating Centre, and the diagnosis was confirmed by three senior clinicians, working independently and unaware of treatment allocation. Women received placebo or vitamin supplementation (vitamin C [1000 mg] and vitamin E [400 IU]), administered on a daily basis from between 8 and 22 weeks gestation until delivery. The DAPIT inclusion and exclusion criteria maternal serum HbA1c, PAI-1 and PAI-II as well as cord vitamin C and E analyses placental nitrotyrosine and vitamin C and E have been described previously (McCance et al., 2010; Johnston, Powell, McCance, Pogue, et al., 2013).

2.2. Placental collection and preparation

Placentae (n = 57) were collected from a DAPIT sub-cohort which comprised of consecutive deliveries in women with type 1 diabetes at the Royal Maternity Hospital Belfast, Northern Ireland by trained study personnel from January 2004 to December 2008. Approval from the West Midlands Multi-Centre Research Committee (MREC/02/7/16) was obtained to allow for placental collection at this single site. Placental collection has been described in detail previously; one placental block (2 cm^3 section) was obtained from both the centre and periphery immediately after delivery (to reduce any inherent structural disparity in placental composition) snap frozen in liquid nitrogen and stored at −20 °C for analyses of antioxidant enzyme activity and lipid peroxidation markers (Johnston et al., 2013).

2.3. Placental antioxidant enzyme activities

A portion of stored placenta was removed and washed in 0.1 M PBS (pH 7.2, 0.15 M NaCl containing 0.5 mM EDTA) then blotted dry. Placental lysates (central and peripheral components) were then prepared for antioxidant enzyme analyses by homogenising placental tissue for 3 min in a 0.1 M PBS, 1 mM EDTA, and 0.05% Triton X solution. Sonication was performed for 10 min on a Lucas Dawe Sonicator (Lucas Dawe Ultrasound, London, UK). Homogenates were vortexed for 10 s and left on ice for 5 min for a total of 2 cycles. Following this, placental lysates were centrifuged at 4472*g* for 20 min at 4 °C. Aliquots of the resulting supernatant were used for antioxidant enzyme assay.

Glutathione peroxidase, glutathione reductase and superoxide dismutase activities were measured using commercially available kits on an ILAB 600 clinical analyser supplied by Instrumentation Laboratory (Lexington, Massachusetts). Glutathione peroxidase activity in placental lysates was assessed by kinetic analysis using a commercially available Ransel kit (catalogue no: RS505), glutathione reductase was also measured by kinetic analysis using a commercially available kit (catalogue no: GR2368) [both supplied by Randox Laboratories Antrim, Northern Ireland]. Gpx and Gred sample absorbance were read at 340 nm. Superoxide dismutase activity in placental lysates was measured by kinetic analysis using a commercially available Ransod kit (catalogue no: SD125), Randox Laboratories and sample absorbance was read at 505 nm. Gpx, Gred and SOD activities were expressed as U/l/mg. Catalase activity in placental lysates was measured by means of a commercially available EIA kit [(catalogue no: 707002), Cayman Chemicals]. Sample absorbance was read at 540 nm on a BioRad Benchmark Microplate reader with data expressed as nmol/min/mg. The intra-assay CV was 1.45% for Gpx, 3.53% for SOD and 1.58% for Gred. The intra-assay CV was 2.4%, and the inter-assay CV was 11.4% for catalase.

2.4. Placental lipid peroxidation

2.4.1. Aqueous phase hydroperoxide

Stored placentae were removed and washed in 0.1 M PBS (pH 7.2, 0.15 M NaCl containing 0.5 mM EDTA) blotted dry. After this, placental lysates were then prepared for aqueous phase hydroperoxide analysis by homogenising placental tissue for 3 min in a 1:100 butylated hydroxytoluene [200 μM; BHT/PBS] solution. Sonication was performed for 10 minutes on a Lucas Dawe Sonicator (Lucas Dawe Ultrasound, London, UK). Placental supernatant was then vortexed for 10 seconds and centrifuged at 4472*g* for 5 minutes at 23 °C. Placental aqueous phase hydroperoxide levels were analysed using the Ferrous Oxidation-Xylenol Orange (FOX-1) assay. Samples were then read at 560 nm on a BioRad Benchmark Microplate Reader with data expressed as μmol/mg. The calculated manual intra-assay CV was 5.1% and the inter-assay CV was 10% for aqueous phase hydroperoxides.

2.4.2. 8-iso-Prostaglandin F2α

Placental lysates were prepared for isoprostane analysis by homogenising placental tissue at 4 °C for 3 min. Following this, 4 M NaOH (sodium hydroxide) was added by volume (1:1; v/v) and heated at 45 °C for 2 hours to ensure hydrolysis. After hydrolysis, samples were cooled at room temperature and treated with an equal volume of 2 M HCl (hydrochloric acid) resulting in a final 1:4 dilution. The neutralised sample were centrifuged at 4472*g* at room temperature for 5 minutes, the pH of the sample was in the range 6–8. Placental 8-iso-prostaglandin F2α was measured by means of a