Oxidative stress increases placental and endothelial cell activin A secretion

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Abstract

Circulating levels of activin A are significantly increased in women with preeclampsia when compared with those with a normal pregnancy. The mechanisms underlying these increased levels are unknown. We undertook these studies to explore whether oxidative stress might be the mechanism. We exposed trophoblast explants, human umbilical vein endothelial cells (HUVECs) and peripheral blood monocytes to oxidative stress in vitro using xanthine/xanthine oxidase (X/XO), measuring activin A and isoprostane in conditioned media and mRNA for activin βA in explants and HUVECs. We also measured isoprostane and activin A in serum from 21 women with preeclampsia and from 20 women with a normal pregnancy. Treatment with X/XO significantly increased 8-isoprostane production from placental explants, HUVECs and monocytes, indicative of oxidative stress, and significantly increased activin A output from placental explants (139.1 ± 27.4 per mg wet weight vs 322.9 ± 89.7 pg/ml per mg wet weight, P = 0.02) and from HUVECs (1.2 ± 0.2 vs 3.2 ± 1.8 ng/ml, P = 0.04). There was no effect on activin A output from monocytes. X/XO significantly increased βA mRNA in placental explants but not in HUVECs. Maternal plasma levels of 8-isoprostane and activin A were significantly higher in women with preeclampsia when compared with controls (333.8 ± 70 vs 176.3 ± 26.2 pg/ml, P = 0.04 and 49.5 ± 7 vs 13.1 ± 1.2 ng/ml, P < 0.001 respectively). In the women with preeclampsia, but not in those with a normal pregnancy, circulating levels of 8-isoprostane and activin A were significantly and positively correlated (r² = 0.72; P < 0.001). These data suggest that oxidative stress may be one of the mechanisms underlying increased circulating activin A in preeclampsia.


Introduction

Activins are dimeric glycoprotein members of the transforming growth factor-β (TGF-β) superfamily (Massague 1990). In pregnancy, the placenta is the major source of activin in the maternal circulation (de Kretser et al. 1994, Qu & Thomas 1995, Wallace & Healy 1996, Fowler et al. 1998), secreting predominantly activin A (Fowler et al. 1998). Maternal serum levels of activin A increase from about mid-pregnancy to a peak close to term (Muttukrishna et al. 1996, Fowler et al. 1998, Schneider-Kolsky et al. 2002), falling quickly after birth (Fowler et al. 1998). In pregnancies complicated by placential dysfunction as evidenced by intrauterine foetal growth restriction (Bobrow et al. 2002, Wallace et al. 2003, Barkehall-Thomas et al. 2006) or preeclampsia (Muttukrishna et al. 1997, D’Antona et al. 2000, Manuelpillai et al. 2001, Silver et al. 2002), maternal serum levels of activin A are significantly higher than observed in normal pregnancy. While these increased circulating levels of activin A are thought to arise from increased placental production (Manuelpillai et al. 2001, Silver et al. 2002) the mechanism(s) underlying such increased placental output remain unclear.

On the basis of histological (Brosens & Renaer 1972) and, more recently, molecular (Soleymanlou et al. 2005) evidence of placential hypoxia in preeclampsia and on the in vivo observations in sheep that both acute (Jenkin et al. 2001) and chronic (Supramaniam et al. 2006) foetoplacental hypoxia increases amniotic fluid levels of activin A, hypoxia has been explored as a possible cause of increased placental activin production in vitro (Blumenstein et al. 2002, Manuelpillai et al. 2003). However, in contrast to the in vivo ovine data, in vitro culture of first trimester and term human placental explants under low oxygen conditions consistently reduced activin A production (Blumenstein et al. 2002, Manuelpillai et al. 2003), suggesting that placental hypoxia is not a likely cause of the increased activin A observed in preeclampsia.

In preeclampsia, while hypoxic (Soleymanlou et al. 2005) the placenta also displays evidence of excessive oxidative stress (Hubel 1999, Burton & Jauniaux 2004, Myatt & Cui 2004), thought to result from either hypoxia-reperfusion injury (Burton & Hung 2003) and/or deficient antioxidant defences (Perkins 2006). In turn, increased placental oxidative stress has been linked to the systemic features of preeclampsia (Roberts & Hubel 1999) through the release of a variety of possible
mediators of endothelial cell dysfunction such as lipid peroxides (Walsh 1998), pro-inflammatory cytokines (Hung et al. 2004, Matthiesen et al. 2005) and syncytiotrophoblast microparticles (Redman & Sargent 2000). Accordingly, we undertook this study to explore whether oxidative stress may be the mechanism underlying increased placental activin A production in preeclampsia and to explore whether circulating levels of activin were correlated with circulating levels of a marker of systemic oxidative stress. Furthermore, since it has been suggested that endothelial cells (Manuelpillai et al. 2001, Tannetta et al. 2003) and leucocytes (Tannetta et al. 2003) may also contribute to circulating levels of activin A, we explored the effect of oxidative stress on activin output from these sources.

Materials and Methods

Sample collection

Maternal blood was collected from the antecubital vein of women with a singleton pregnancy in the absence of labour. Samples were collected from 21 women between 26 and 40 weeks of pregnancy with established preeclampsia, as defined by Australian Society for the Study of Hypertension in Pregnancy (Brown et al. 2000), medicated with oral labetalol and/or nifedipine and from 20 women with a gestation-matched healthy pregnancy. Blood was centrifuged at 2000 g for 10 min at room temperature for serum collection and at 4 °C for plasma. Serum was stored at −20 °C prior to analysis. To prevent oxygen free radical formation 0.005% butylated hydroxytoluene (BHT, Sigma) was added to plasma samples prior to storage at −80 °C.

Placental tissue (n = 11), umbilical cords (n = 6) and maternal peripheral blood (n = 6) for explant and cell isolation were obtained from women with a healthy, singleton pregnancy undergoing an elective caesarean section at term (37–40 weeks gestation) for either breech presentation or previous caesarean section.

All women participating in the study gave informed written consent. The study was approved by the Southern Health Human Research and Ethics Committee.

Placental explant culture

Explants (35–50 mg) from terminal regions of chorial vili were coated in the ratio of 1:3 dilution (v/v) Matrigel (BD Biosciences, Franklin Lakes, NJ, USA) and placed in six-well culture dishes (BD Biosciences). Each well contained approximately 150 mg tissue. Explants were cultured in serum free DMEM/F12 (Gibco-BRL) at 37 °C in 5% CO₂ and 95% air for 48 h before treatment, as previously described (Manuelpillai et al. 2003). Explants were then treated with 2·3 mM xanthine (X) and 0·015 U/ml xanthine oxidase (XO) (Sigma), as previously described by others (Malek et al. 2001) for a further 24 and 48 h. Untreated cultures served as controls. For antioxidant treatment, cells were incubated with X/XO (as above) for 24 h and then treated with X/XO and 100 U/ml superoxide dismutase (SOD; Khullar et al. 2004) or 50 μM vitamin C and 25 μM vitamin E (Miczarek et al. 2000) for a further 24 h before media collection and RNA isolation. Conditioned media were collected and stored at −20 °C for activin A and 8-isoprostane analysis. Explants were snap frozen and stored at −80 °C for RNA extraction.

Human umbilical vein endothelial cells (HUVECs) isolation and culture

HUVECs were isolated as previously described (Jaffe et al. 1973). Cells were cultured in M199 medium (Gibco-BRL) with 20% heat-inactivated FCS, epidermal and fibroblast growth factors (10 ng/ml each), heparin (90 μg/ml), penicillin G (100 U/ml), streptomycin sulphate (100 μg/ml) and amphotericin B (0·25 μg/ml; Invitrogen). HUVECs were seeded into flasks coated with 0·2% gelatin (Sigma-Aldrich). Cells were expanded and cultures used for experiments were taken from second or third passages.

Cells were seeded in 24-well plates (5 × 10⁴ cells/well) coated with gelatin and maintained in culture for 48 h prior to treatment. To induce oxidative stress, media were supplemented with 50 μM X and 0·8 mU/ml XO (Sigma) and conditioned media collected and stored at −20 °C after 24 and 48 h for subsequent activin A and 8-isoprostane analysis. The doses of X/XO were based upon dose-response studies utilising 8-isoprostane as an outcome (data not shown). The antioxidant treatment was as described above before media collection and RNA isolation.

Monocyte isolation and culture

Monocytes were isolated from peripheral blood mononuclear cells (PBMCs) after Ficoll-Paque Plus (Amersham Biosciences) density configuration, using magnetic cell sorting (MACS), (Miltenyi Biotec, Gladbach, Germany) as previously described (Hafsi et al. 2004). Cells (2 × 10⁶ cells/ml) were plated in 24-well plates and cultured in RPMI 1640 supplemented with antibiotics, 10% FCS, 50 μM X and 0·8 mU/ml XO for 24 h. The antioxidant treatment was as described above. Conditioned media were collected after 24 and 48 h and stored at −20 °C for activin A and 8-isoprostane analysis.

Quantitative real-time PCR

Total RNA was isolated from placental explants and HUVECs using Trizol reagent (Invitrogen) and cell to signal lysis buffer (Ambion, Austin, TX, USA) respectively. Two and a half micrograms of total placental RNA and 12 μl HUVEC lysate were reverse transcribed into cDNA (cDNA) using random primers and Superscript III (Invitrogen). Placental cDNA was diluted in the ratio of 1:100. Five microlitres of cDNA were mixed with primers specific for human activin A (that spanned intron–exon junctions), SYBR Green reaction mix (Roche Diagnostics) and subjected to quantitative real-time PCR (q-PCR, Rotor Gene RG-3000, Corbett Research, Sydney, www.endocrinology-journals.org

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Oxidative stress increases activin A  ·  S Mandang and others 487

Australiа). The forward and reverse activin A primers were 5′ GGA GGG CAG AAA TGA ATG AA 3′; 5′ CTG CTG GAG ACA GGG AAG AC 3′ respectively. The cycling parameters were: denaturation 95 °C for 10 s, annealing 59 °C for 15 s and extension 72 °C for 15 s. Using these parameters, a single peak melt curve for activin βA was obtained with a melt temperature of 90 °C. Agarose gel electrophoresis was performed to check the size of the amplicon generated, confirming it to be 350 bp consistent with its predicted size, and the product verified as activin βA by DNA sequencing. For normalisation, 2 μl cDNA were amplified to detect 18S rRNA alongside the target cDNA. Forward and reverse 18S primers were 5′ CGG CTA CCA CAT CCA AGG AA 3′; 5′ GCT GGA ATT ACC GCG GCT 3′ respectively. The primers used to amplify 18S rRNA gave a single peak melt curve with a melt temperature of 87 °C. The amplicon generated was 180 bp in length and its sequence was also verified. Negative controls reactions for βA and 18S lacked melt curves. The amplification efficiencies for activin βA and 18S were 1.04 and 1.02 respectively, determined using Corbett Instruments Rotorgene software. Standard curves for activin A and 18S were prepared from term placental cDNA and the cDNA concentrations relative to 18S was determined using Rotor Gene version 6.0 software (Corbett Research). For each test sample, the concentration of βA, determined using the Rotorgene software, was divided by the concentration of 18S for purposes of normalisation. For each sample, analyses were carried out in duplicate and averaged.

Activin A ELISA

Total activin A in maternal serum and conditioned media was measured using a commercial two-site enzyme-linked immunosorbant assay (DSL, Webster, TX, USA), with minor modifications as described previously (Riley et al. 1998). Maternal serum and placental explant conditioned media were diluted in the ratio of 1:10 and 1:3 with assay diluent respectively, whereas cell conditioned media was assayed undiluted. The sensitivity of the assay was 72 pg/ml and the mean intra- and inter-assay coefficients of variation were 8 and 15% respectively.

8-Isoprostane EIA

8-Isoprostane in maternal plasma and conditioned media was measured using a commercial enzyme immunoassay (Cayman Chemical, Ann Arbor, MI, USA) according to the manufacturer’s instructions. Plasma was assayed undiluted, whereas placental explant and cell conditioned media were diluted in the ratio of 1:100 and 1:5 respectively. The intra- and inter-assay coefficients of variation were 7-2 and 18-5% respectively. The sensitivity of the assay was 5 pg/ml.

Statistical analysis

All the data are expressed as means ± S.E.M. Differences in maternal serum levels of activin A and 8-isoprostane between women with preeclampsia and a normal pregnancy were analysed using unpaired t-test. Correlations between activin A and 8-isoprostane in both groups were assessed by Pearson’s rank correlation coefficient. The effect of X/XO on placental explant, HUVEC and monocyte activin A secretion was assessed by paired t-test. Kruskal–Wallis test followed by post hoc Dunn’s test for multiple comparisons were used to analyse the differences in mRNA expression and one-way ANOVA and post hoc Bonferroni’s test for multiple comparisons were used to analyse the changes in placental explant and HUVEC activin A output in the presence of SOD and vitamin C/E. All statistical analyses were performed using GraphPad Prism version 4.01 (San Diego, CA, USA). In all cases significance was assumed when P<0.05.

Results

Activin A and 8-isoprostane in maternal circulation

The clinical characteristics of the 41 women with preeclampsia and normal pregnancy at the time of maternal blood collection are detailed in Table 1. Maternal plasma levels of 8-isoprostane were significantly higher in women with preeclampsia when compared with controls (333.82 ± 70.01 vs 176.32 ± 26.24 pg/ml, P=0.04) as were maternal serum levels of activin A (49.46 ± 7.02 vs 13.13 ± 1.84 ng/ml, P<0.001). In women with preeclampsia there was a significant positive correlation between circulating levels of 8-isoprostane and activin A (Fig. 1A, Pearson’s correlation r²=0.72; P<0.001) but no correlation in women with a normal pregnancy (Fig. 1B, Pearson’s correlation r²=−0.15; P=0.17).

Effect of X/XO on 8-isoprostane, activin A secretion and activin βA mRNA expression

X/XO treatment induced oxidative stress in placental explants, HUVECs and monocytes as shown by increased levels of 8-isoprostane when compared with controls (Table 2).

Table 1 Clinical characteristics of women with preeclampsia and normal pregnancy. Data are presented as means ± S.E.M.

<table>
<thead>
<tr>
<th></th>
<th>NP (n=20)</th>
<th>PE (n=21)</th>
<th>P value</th>
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<tbody>
<tr>
<td>Maternal age (years)</td>
<td>32.4±1.1</td>
<td>29.8±1.3</td>
<td>0.01</td>
</tr>
<tr>
<td>Gestational age at</td>
<td>31.2±0.8</td>
<td>33.4±1.0</td>
<td>0.01</td>
</tr>
<tr>
<td>sampling (weeks)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gestational age at</td>
<td>37.2±0.6</td>
<td>33.8±1.0</td>
<td>0.04</td>
</tr>
<tr>
<td>delivery (weeks)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primigravida (%)</td>
<td>65</td>
<td>67</td>
<td>0.05</td>
</tr>
<tr>
<td>Systolic BP (mmHg)</td>
<td>109.8±2.1</td>
<td>158.2±4.4</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Diastolic BP (mmHg)</td>
<td>70.6±2.0</td>
<td>101.8±2.1</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>BMI (kg/m²)</td>
<td>25.2±0.9</td>
<td>26.4±1.6</td>
<td>0.7</td>
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NP, normal pregnancy; PE, preeclampsia; BP, blood pressure; BMI, body mass index.
When compared with control cultures, treatment of placental explants with X/XO significantly increased activin A in conditioned media (Fig. 2A: mean ± S.E.M. activin A: 1·1 ± 0·2 vs 2·1 ± 0·2 ng/ml at 24 h, P = 0·01 and 1·2 ± 0·2 vs 3·2 ± 1·8 ng/ml at 48 h, P = 0·04). Basal levels of activin A in monocyte-conditioned media were very low and did not increase in the presence of X/XO (Fig. 2C). Consistent with the changes in activin A in conditioned media, treatment with X/XO significantly increased the expression of activin βA mRNA in placental explants at 24 h (P = 0·03 when compared with control; Fig. 3A). There were no significant differences in activin βA mRNA expression in either HUVECs (Fig. 3B) or monocytes (data not shown).

**Table 2** Mean ± S.E.M. levels of 8-isoprostane in media conditioned by placental explants (pg/mg wet weight tissue), HUVECs (pg/ml) and peripheral blood monocytes (pg/ml) under control conditions and in the presence of X/XO.

<table>
<thead>
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<th></th>
<th>48 h</th>
<th>24 h</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Placental explants</td>
<td>Control: 11·8 ± 2·9</td>
<td>X/XO: 40·9 ± 10·9</td>
<td>0·009</td>
</tr>
<tr>
<td>HUVECs</td>
<td>Control: 119·9 ± 37·4</td>
<td>X/XO: 271·4 ± 28·5</td>
<td>0·03</td>
</tr>
<tr>
<td>Monocytes</td>
<td>Control: 270 ± 46·8</td>
<td>X/XO: 375·2 ± 51·6</td>
<td>0·14</td>
</tr>
</tbody>
</table>

**Effect of antioxidants on X/XO induction of 8-isoprostane, activin A secretion and activin βA mRNA expression**

The addition of vitamin C/E, but not SOD, significantly mitigated X/XO-induced release of 8-isoprostane at 24 h from placental explants (P = 0·02, Fig. 4A). There was no apparent effect of either vitamin C/E or SOD on X/XO-induced 8-isoprostane release from HUVECs (Fig. 4B).

The addition of vitamin C/E, but not SOD, significantly mitigated both X/XO-induced release of activin A from placental explants (P < 0·01, Fig. 5A) and X/XO-induced activin βA mRNA expression in placental explants (P < 0·04, Fig. 3A) at 24 h. The effects of vitamin C/E and SOD treatment on X/XO-induced changes in activin βA mRNA expression and activin A secretion from HUVECs were not statistically significant (P = 0·96 and 0·55 respectively; Figs 3B and 5B).

**Discussion**

In this study, we have shown that oxidative stress, induced by X/XO, stimulates activin A production and secretion from placental explants and endothelial cells but not from peripheral blood monocytes. We have further shown that in women with preeclampsia circulating levels of activin A are significantly associated with 8-isoprostane, a marker of lipid peroxidation and excessive systemic oxidative stress. Taken together, these new observations suggest that oxidative stress may be a mechanism underlying the increased levels of activin A present in women with preeclampsia.
Figure 2 The effect of X/XO on term placental explant (A), HUVEC (B) and monocyte (C) secretion of activin A in vitro. ns, not significant.

Figure 3 The effect of X/XO and antioxidants (SOD and vitamin C/E) on median activin βA mRNA expression in placental explants (A) and HUVECs (B). ns, not significant.
As reviewed elsewhere (Tong et al. 2003), circulating levels of activin A are greatly increased in women with preeclampsia when compared with those with a normal pregnancy. Previous studies have shown that the placental content and circulating levels of activin A in preeclampsia are closely correlated (Manuelpillai et al. 2001) and that circulating activin A levels fall very quickly after birth (Fowler et al. 1998). These observations are consistent with the placenta being the major source of circulating activin A in both normal pregnancy and preeclampsia. Nonetheless, in this study, we have also confirmed previous reports that endothelial cells produce activin A (Manuelpillai et al. 2001, Tannetta et al. 2003), suggesting that the endothelium may be another source of circulating activin A in preeclampsia. In contrast, while we were also able to confirm that monocytes produce activin A in vitro (Tannetta et al. 2003) our data suggest that these cells produce very little activin and that they do not respond to oxidative stress. Accordingly, we believe that circulating monocytes are not likely to be a major source of high activin A levels in women in preeclampsia. Further, if monocyte production of activin is increased in preeclampsia

**Figure 4** The effect of X/XO and antioxidants (SOD and vitamin C/E) on 8-isoprostane secretion from placental explants (A) and HUVECs (B) in vitro at 24 h. ns, not significant.

**Figure 5** The effect of X/XO and antioxidants (SOD and vitamin C/E) on activin secretion from placental explants (A) and HUVECs (B) in vitro at 24 h. ns, not significant.
(Tannetta et al. 2003), then the mechanism underlying this is most likely via inflammatory cytokines (Tannetta et al. 2003) and not increased oxidative stress.

Whatever the relative contributions of the placenta and the endothelium to circulating activin, until now the mechanism(s) underlying the heightened levels of activin observed in preeclampsia have been obscure. Here, we have shown that oxidative stress, induced by X/XO, significantly increases the production and secretion of activin A from both placental explants and endothelial cells in vitro. We chose X/XO to induce oxidative stress as this is thought to be one of the mechanisms underlying ischaemia-reperfusion injury in the placenta in preeclampsia (Hung et al. 2001). The addition of X/XO results in the increased production of $O_2^-$ in the first instance and thereafter peroxidative damage. The mitigation of this effect by the antioxidants vitamin C and E confirms that oxidative stress was involved although precisely which reactive oxygen species are acting to increase activin has not been explored completely. That SOD did not mitigate the effects of X/XO was surprising, as SOD exerts its antioxidant effects via scavenging $O_2^-$.

It is also unclear whether oxidative stress increases activin directly or indirectly in vitro. In this regard, oxidative stress is known to activate the transcription factor NF-$k_B$, which in turn induces a number of pro-inflammatory cytokines (Li & Karin 1999). This is relevant because activin release from a number of cell types, including trophoblast cells, is stimulated by inflammation (Keelan et al. 2000, Jones et al. 2004). Indeed, recent ovine studies have suggested that, together with TNF-$\alpha$, activin release is one of the very first responses to systemic inflammation significantly preceding elevations in other inflammatory cytokines such as interleukin-6 (Jones et al. 2004). Thus, in the context of preeclampsia, which is essentially an exaggerated systemic inflammatory disease (Sibai et al. 2005) triggered, at least in part, by oxidative placental damage (Hubel 1999, Burton & Jauniaux 2004, Myatt & Cui 2004), it is very likely that excessive placental and/or endothelial oxidative stress, induced inflammation leads to increased activin production (Manuelpillai et al. 2001, Silver et al. 2002). Further studies are required to define, whether the effects of oxidative stress on activin transcription and production are NF-$k_B$-dependent.

It is possible that the control condition of 20% $O_2$, used in this study, which is physiologically hyperoxic, is itself increased activin output through relative oxidative stress. Indeed, previous in vitro studies using placental explants have shown that reduced $O_2$ reduces activin production (Blumenstein et al. 2002, Manuelpillai et al. 2003). In those studies activin output from placental explants in a ‘low’ oxygen environment was compared with output in air (20% $O_2$). The findings of this current study suggest that rather than ‘low’ oxygen reducing placental activin secretion, the control conditions of 20% $O_2$, which is physiologically hyperoxic, is likely to have artificially increased activin output through relative oxidative stress. In light of this current study, it would be worthwhile revisiting the previous work with supplemental antioxidants in the culture media. This would be expected to assist with understanding the relative roles of early placental hypoxia–hyperoxia in the pathogenesis of preeclampsia (Hubel 1999). With regard to the current study, it is possible that culture of trophoblast in 20% $O_2$, itself induces oxidative stress prior to treatment with X/XO or at least augments the effect of X/XO due to the increased availability of $O_2$, which is needed to generate $O_2^-$. In this regard, as with any in vitro culture data, care should be taken when extrapolating these in vitro findings to in vivo mechanisms.

Nonetheless, the observation here that circulating levels of activin A and 8-isoprostane were strongly correlated in women with preeclampsia, but not in women with a normal pregnancy, is consistent with our interpretation of the in vitro data that oxidative stress is the mechanism leading to increased activin secretion. Eight-isoprostane is thought to be a sensitive and stable marker of oxidative stress, derived from cell membrane phospholipids by free radical peroxidation of arachidonic acid (Morrow & Roberts 1996, Roberts & Morrow 2000). Circulating levels of 8-isoprostane are increased in preeclampsia (McKinney et al. 2000). The apparent relationship between activin and isoprostane suggests that in any given individual the circulating level of activin A may reflect the severity of systemic oxidative damage, offering the potential use of activin as a marker of disease progression and/or response to therapy. Of course, in this current study we have not assessed whether the treatment of preeclampsia alters activin A levels, but the recent report that antioxidant treatment suppresses oxidative stress and the subsequent inflammatory response in placental explants suggests that this would be worthy of study (Cindrova-Davis et al. 2006). Further, in women who subsequently develop preeclampsia circulating activin A levels increase in early pregnancy, long before clinical signs or symptoms (Muttukrishna et al. 2000). This early increase in activin may be indicative of early placental oxidative damage affording further insights into the timing and progress of the pathogenesis of preeclampsia.

In summary, we have shown that oxidative stress is a possible mechanism underlying increased activin A production from the placenta and/or endothelium in preeclampsia and that activin may be a useful marker of systemic oxidative damage.

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