Potential Role of Glucocorticoids in the Pathophysiology of Intrauterine Growth Restriction (IUGR)

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Abstract

Although the etiology of intrauterine growth restriction (IUGR) and preeclampsia (PE) remains unclear, most investigators attribute the initial "insult" to poor utero-placental perfusion due to defective trophoblast invasion that ultimately compromises fetal well-being.\(^1\) The resultant hypoxia curtails the remodeling of uterine vessels by invasive cytotrophoblasts in the second trimester.\(^1,2\) Our results suggest that mediators of fetal stress [i.e., glucocorticoids (GC)] may in fact alter placental gene expression and contribute to the destruction of the placental villous network in pregnancies with IUGR/PE. We will present a molecular model through which GC, induced in response to fetal stress, promotes the placental villous damage observed in pregnancies associated with IUGR/PE. This model incorporates the roles of trophoblast plasminogen activator inhibitor (PAI)-1, mesenchymal extracellular matrix (ECM) proteins, and their regulation by transforming growth factor (TGF)-β. We will employ the term "IUGR/PE" to describe those pregnancies with severely growth-restricted fetuses may also complicated by maternal PE. These conditions frequently coexist, and a review of the literature suggests that this placental pathology may be associated with both IUGR and PE. Furthermore, considerable attention has been given to the role of exogenously administered and stress-induced endogenous increases in fetal GC and the development of IUGR. There is mounting evidence that aberrant elevations in GC during fetal life and/or IUGR may result in fetal programming of chronic diseases of adulthood such as diabetes, coronary artery disease, and hypertension.

Excess Placental Fibrin and ECM Proteins Are Noted in Pregnancies with IUGR/PE

The most severe cases of IUGR/PE are associated with 40 to 50 percent fetal mortality and are usually characterized by absent or reversed end diastolic flow (AEDF or REDF) in the umbilical artery.\(^4\) Placentas delivered from pregnancies with AEDF show a higher frequency of maldeveloped, elongated, poorly branched, and poorly vascularized terminal villi, the principal sites of nutrient and oxygen exchange between mother and fetus.\(^5,6\) Although the precise etiology of these changes in placental structure is not known, it is generally thought to result from defective cytotrophoblast invasion in the first two trimesters of pregnancy.\(^1,3\) Histological studies of IUGR/PE placentas have revealed two specific biochemical changes relative to controls matched for gestational age; excessive perivillous (i.e., in the intervillous space) deposition of fibrin and up-regulation of ECM proteins in the villous core.\(^7-9\) Immunohistochemical
analyses have demonstrated thickened basal lamina and increased expression of collagen III and IV and laminin in the core of the placental villus in pregnancies with IUGR/PE. In this same study, electronmicroscopy identified placental mesenchymal cells (PMCs) as the likely source of enhanced ECM protein expression noted in IUGR/PE placentas. Furthermore, in pregnancies with IUGR/PE, excessive perivillous deposition of fibrin and intravillous ECM proteins was associated with extensive placental villous damage (increased prevalence of white infarcts or collapsed villi, necrosis, and fibrosis) and impairment of nutrient transport. Thus, it is likely that hyper-accumulation of perivillous fibrin and placental ECM proteins in pregnancies associated with IUGR/PE disrupts placental architecture, collapses the villous network, and irreversibly restricts the flow of nutrients between mother and fetus (Fig. 1). We do not propose that excessive deposition of fibrin and ECM proteins cause IUGR, but rather that they play a critical role in the pathophysiology of these pregnancies. In addition, we acknowledge that there are several other potential cellular mechanisms of placental damage in pregnancies with IUGR and PE including apoptosis, hypoxia and reperfusion injury, all of which may be affected by GC. However, for the purpose of this report we are limiting our discussion to a potential unifying mechanism underlying excessive placental fibrin and ECM protein deposition in these pregnancies.

**Plasminogen Activator Inhibitor (PAI-1): Role in Fibrin Deposition in Pregnancy**

PAI-1 is a 52 kD protein that is a member of a serpin (serine protease inhibitor) family of protease inhibitors that also includes PAI-2 and PAI-3. PAI-1 is the primary inhibitor of fibrinolysis based on its high affinity suppression ($K_i = 1$ nM) of tissue type plasminogen activator (tPA). PAI-1 forms a 1:1 molecular complex with tPA and inhibits tPA-mediated conversion of plasminogen to plasmin, the major fibrinolytic factor. PAI-1 was originally described as an endothelial cell protein, but later reports revealed PAI-1 to be synthesized by many cell types, including those found at the uterine-placental interface, e.g., trophoblasts and decidual cells. Interaction with the ECM protein vitronectin stabilizes PAI-1 and may localize it to areas of thrombosis. Excessive production of PAI-1 would be expected to compromise fibrin clearance when the clotting cascade is activated. Pregnancy is considered to be a thrombophilic state based on the elevation of several plasma coagulation factors (e.g., factor VII, VIII, X and fibrinogen) in maternal sera across gestation. 
Several lines of evidence implicate placental PAI-1 in the excessive levels of perivillous fibrin deposition and placental damage noted in pregnancies with IUGR and PE. Levels of PAI-1 in maternal sera from IUGR/PE pregnancies are elevated, and plasma levels of PAI-1 correlated with the severity of placental damage. Elevated levels of PAI-1 mRNA (by Northern blotting) and protein (by immunohistochemistry) are observed in IUGR/PE placentas compared to gestational age-matched controls. Syncytiotrophoblasts (SCTs), the placental cell type that line the intervillous space and are in direct contact with maternal blood, were shown by in situ hybridization to be the likely source of elevated placental PAI-1 in pregnancies associated with IUGR/PE. In uncomplicated pregnancies, PAI-1 expression is limited to extravillous and invasive trophoblasts and not SCTs, indicating that syncytial expression of PAI-1 is pathological. Further evidence for a role of PAI-1 in extravillous trophoblast function is the recent result showing that the presence of function blocking anti-PAI-1 antibodies promoted invasiveness of human first trimester extravillous trophoblasts. Of note, infarcted areas of IUGR/PE placentas have been demonstrated to manifest the highest levels of immunoreactive PAI-1 in SCTs. In contrast, lower levels of PAI-2 were found in placentas and maternal sera in pregnancies with IUGR/PE compared to controls. Compared to PAI-2, PAI-1 is 1000-fold more inhibitory of tPA-mediated fibrinolysis. These observations indicate that PAI-1 is the dominant regulator of fibrinolysis in the intervillous space in pregnancies with IUGR/PE. These results suggest that the localization as well as the elevation of placental PAI-1 is important in the genesis of placental damage and thrombotic sequelae associated with IUGR/PE.

Role of TGF-β and Hypoxia on the Expression of PAI-1 and ECM Proteins

TGF-β, is a cytokine family of three closely related peptides (TGF-β1, β2, β3), that act through membrane receptors (types I and II). Downstream signaling of TGF-β is mediated by phosphorylated Smads (Sma and MAD gene homologues in C. elegans and D. melanogaster) that bind to TGF-β-responsive 5' upstream sequences. Smad binding sites have been identified in the promoters of genes including PAI-1. The 3 TGF-βs and types I and II receptors are expressed by human placenta. Early studies showed that TGF-β treatment stimulated the synthesis of ECM proteins including fibronectin, collagens and their cell surface integrin receptors. TGF-β was later found to be an important enhancer of PAI-1 expression in several cell types including HTR-8/SVneo cells, a first trimester cytotrophoblast cell line. Autocrine regulation of first trimester cytotrophoblast proliferation and invasion by TGF-β has been proposed.

Hypoxia up-regulates gene transcription through the binding of hypoxia inducible factor (HIF)-1 to hypoxia response elements (HRE) in the promoter region of several genes including erythropoietin, heme oxygenase-1 and vascular endothelial cell growth factor. HRE-like sequences have been identified in the PAI-1 promoter. Graham and colleagues showed that both hypoxia and TGF-β up-regulate PAI-1 expression in HTR-8/SVneo cells. Using an antibody that blocks the action of TGF-β1 and β2 they showed that the effects of hypoxia on PAI-1 expression were not mediated by TGF-β, an important finding in light of previous studies showing that hypoxia up-regulates TGF-β expression in several cell types. Hypoxia, like TGF-β, also enhances the expression of ECM proteins including collagen I, III and IV in lung parenchymal cells, and collagen IV and fibronectin in mesangial cells, and collagen I in cardiac fibroblasts.

Evidence That Glucocorticoids Stimulate PAI-1 and ECM Protein Expression in Placenta by Enhancing the Action of TGF-β

As reviewed above, excessive production of placental PAI-1 has been associated with aberrant periplacental fibrin deposition in pregnancies complicated by preeclampsia PE and IUGR. We recently employed HTR-8/SVneo cells and primary cultures of term cytotrophoblasts as
models for study of PAI-1 regulation by transforming growth factor (TGF)-β, and dexamethasone (DEX), a synthetic GC. ELISA and assays revealed that DEX treatment significantly enhanced TGF-β effects on PAI-1 protein levels culture medium of HTR-8/SVneo cells and cytotrophoblasts several fold (Figs. 2 and 3). Conversely, DEX and TGF-β treatment suppressed PAI-2 levels in HTR-8/SVneo cells and did not affect PAI-2 levels in cytotrophoblasts. This indicated that the effects of DEX and TGF-β on PAI-1 expression in trophoblasts were specific. This result is interesting, because unlike PAI, PAI-2 is not suggested to be a critical

Figure 2. Regulation of PAI-1 and PAI-2 levels in HTR-8/SVneo cells by DEX and TGF-β. HTR-8/SVneo cells were maintained for 48 or 96 h in serum-free medium without (C) or with 100 nM DEX (D) and 1 ng/ml TGF-β (T) and levels of PAI-1 (Panel A) and PAI-2 (Panel B) in culture media were measured by ELISA in 5 independent experiments. Results are expressed as a mean ± SEM. *= P<0.05 vs control; **= P<0.05 vs the 3 other treatment groups (from ref. 64).

Figure 3. Regulation of PAI-1 expression in cytotrophoblasts by DEX and TGF-β. Primary cultures of cytotrophoblasts were maintained for 48 or 96 h in serum free medium without (C) or with 100 nM DEX (D) and 1 ng/ml TGF-β (T). Levels of PAI-1 (Panel A) and PAI-2 (Panel B) in culture media were measured by ELISA from cells isolated from 4 and 5 different placentas, respectively. Results are expressed as a mean ± SEM. *= P<0.05 vs control; **= P< 0.05 vs the 3 other treatment groups (from ref. 64).
Figure 4. Regulation of PAI-1 promoter activity in HTR-8/SVneo cells by DEX and TGF-β. Transfected cells were maintained for 48 h in serum-free medium without (C) and with 100 nM DEX (D) and 1 ng/ml TGF-β (T). Levels of PAI-1 luciferase activity in cell extracts were determined and normalized to Renilla luciferase. Results are expressed as a mean ± SEM from 4 independent experiments. *= P< 0.05 vs control (from ref. 64).

regulator of fibrinolysis, but rather may perform an important anti-apoptotic role. Northern blotting analyses have demonstrated that DEX stimulated levels of PAI-1 mRNA in HTR-8/SVneo cells and cytotrophoblasts several-fold. Unexpectedly, PAI-1 promoter assays have revealed that TGF-β, but not DEX, enhanced PAI-1 expression in HTR-8/SVneo cells through a transcriptional mechanism, since DEX treatment suppressed PAI-1 promoter activity under both basal and TGF-β-stimulated conditions (Fig. 4). These results suggest that GC may alter fibrinolytic and invasive properties of trophoblasts through enhancing TGF-β effects on PAI-1 expression.

To elucidate potential mechanisms of ECM protein regulation in the stroma of the placental villus, we recently examined the interaction of GC and TGF-β in the modulation of ECM proteins in cultures of placental mesenchymal cells (PMCs, i.e., fibroblasts) isolated from human term placentas. Initial results obtained by ELISA showed that, similar to the effects observed for PAI-1 above, the combined treatment with dexamethasone (DEX) and TGF-β enhanced oncofetal fibronectin (FFN) protein levels in serum-free culture medium several fold (Fig. 5). FFN was chosen as a model for study since it is a major ECM protein synthesized in vivo and in vitro by PMCs. Real-time PCR analyses revealed a similar enhancement in levels of FN mRNA in cells treated with TGF-β and DEX (Fig. 6). Real-time PCR results have specifically demonstrated that DEX and TGF-β enhanced collagen (Col) I and Col IV expression, but did not affect levels of Col III or laminin, which is strongly suggestive of selective stimulation of ECM proteins. Similar results were obtained by Northern blotting. In marked contrast to the results obtained with PMCs, we noted that DEX treatment suppressed FFN levels in untreated and TGF-β-treated cytotrophoblasts (Fig. 7), suggesting that GC and TGF-β modulate FFN expression in placenta in a cell type-specific manner. This indicates that GC and TGF-β may be key regulators of ECM protein synthesis in PMCs.

We predict that pregnancies associated with excessive placental exposure to GC and TGF-β would result in over-expression of ECM proteins by PMCs. Placentas from pregnancies with IUGR showed excessive villous fibrosis and ECM production by PMCs. IUGR pregnancies typically have elevated umbilical cord Dopplers or absent diastolic flow which is indicative of increased placental vascular resistance. The enhanced ECM protein production by PMCs that we have demonstrated in our study, and the resulting villous fibrosis seen on histological
Figure 5. Effect of DEX and TGF-β treatments on oncofetal fibronectin (FFN) levels in placental mesenchymal cells (PMCs). PMCs were maintained for 48 or 96 h in serum-free medium without (designated "C" for control) and with 100 nM DEX (designated "D") and 1 ng/ml TGF-β (designated "T"). Levels of FFN in the culture medium were measured by ELISA and were normalized to cell protein. Results are presented as a mean ± SEM obtained in 4 independent experiments. *P < 0.05 vs all other groups by ANOVA (from ref. 67).

Figure 6. Analysis of DEX and TGF-β effects on ECM protein expression in PMCs by real-time PCR. Cells were maintained for 48 h in serum free-medium under two conditions: without (unfilled bars) or with 100 nM DEX and 1 ng/ml TGF-β (filled bars). RNA was extracted from cell lysates, converted to cDNA and levels of FN, laminin (LN), Col I, III, and IV expression were quantitated by real-time PCR analysis. Results are expressed as fold change relative to the calibrator (the lowest control value in each experiment), and are presented as a mean ± SEM obtained in 5 independent experiments. *P < 0.05, **P < 0.01, ***P < 0.001 by Student's t test (from ref. 67).
Figure 7. DEX and TGF-β effects on FFN levels in cultures of cytotrophoblasts (CTs). CTs were maintained for 48 h in serum-free medium in the absence (C) or presence of 100 nM DEX (D) and 1 ng/ml TGF-β (T) and the level of FFN in culture media was assessed by ELISA. Results are presented as a mean ± SEM from 6 culture wells obtained in a single experiment representing 3 identically conducted ones. *P < 0.01 vs control, **P < 0.01 vs control and TGF-β-treated group by ANOVA (from ref. 67).

examination, may contribute to the clinical observation of increased placental vascular resistance. These changes in the villous stroma have been suggested to reduce the flow of nutrients from mother to fetus in pregnancies with IUGR.

The level of GC in fetal sera has been found to be higher in pregnancies with IUGR compared to appropriately grown gestational age-matched controls. The source of elevated periplacental GC in these pregnancies has been attributed to reduced placental levels of 11β-hydroxysteroid dehydrogenase-2 (the enzyme that irreversibly converts cortisol to the receptor inactive cortisone). In addition, a direct correlation has been noted between the number of doses of antenatal GC given to women at risk for preterm delivery and the severity of villous fibrosis.

Model of the Role of GC in Placental Damage in Pregnancies with IUGR/PE

Taken together, these studies suggest that the combined actions GC and TGF-β may play a critical role in the placental pathophysiology in pregnancies complicated by IUGR/PE. The model presented in Figure 8 proposes that reduced uterine-placental perfusion (hypoxia) initially activates stress pathways in the fetus leading to elevated levels of cortisol in fetal sera. Elevated periplacental levels of GC would then be expected to lead to excessive levels of fibrin and ECM proteins by specifically enhancing the effects of TGF-β on PAI-1 levels in trophoblasts and ECM proteins in PMCs, respectively. These changes would then promote villous collapse (infarction) and a further reduction in the flow of nutrients from mother to fetus.

Recent clinical trials have demonstrated that sustained exposure of the fetus and newborns to exogenous GC have been associate with IUGR. Furthermore, prenatal exposure of the fetus and newborns to exogenous GC in animal models have demonstrated decreased somatic growth of the fetus, reduced glomerular numbers, and decreased islet cell numbers in the...
Figure 8. Model for the role of GC in placental pathophysiology in pregnancies complicated by IUGR/PE. We suggest that elevated periplacental concentrations of GC due to fetal stress in pregnancies with IUGR/PE would promote excess fibrin and ECM protein expression in placenta through the enhancement of the actions of TGF-β. The resulting fibrosis and fibrin deposition is postulated to collapse the villous network resulting in severe placental damage. The reduction in the transfer of oxygen and nutrients from mother to fetus promotes IUGR. In addition, prenatal exposure of the fetus and newborn to IUGR and GC has been suggested to program the fetus for hypertension (HTN) and other chronic diseases as adults.

Fetal pancreas, all of which support the hypothesis of fetal programming of chronic diseases such as hypertension and diabetes. Therefore, GC appears to play a central role in the pathophysiology of IUGR and the programming of some major chronic adult diseases during fetal life.

References


45. Mitchell EJ, O’Connor-McCourt MD. A transforming growth factor beta (TGF-P) receptor from human placenta exhibits a greater affinity for TGF-P2 than for TGF-P1. 1998; Biochemistry 30:4350-4356.


63. Agocha A, Lee HW, Eghbali-Webb M. Hypoxia regulates basal and induced DNA synthesis and collagen type I production in human cardiac fibroblasts: Effects of transforming growth factor-β1, thyroid hormone, angiogenin II and basic fibroblast growth factor. J Mol Cell Cardiol 1997; 29:2233-2244.

64. Ma Y, Ryu JS, Dulay A et al. Regulation of plasminogen activator inhibitor-1 (PAI-1) expression in a human trophoblast cell line by glucocorticoid and transforming growth factor (TGF)-β. Placenta 2002; 23:727-734.


