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Placental Expression of Vasoactive Agents and Tissue Factor in Pre-Eclamptic and Clyocosporine Treated Kidney Transplant Mothers

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Abstract

We studied the Endothelin-1 (ET-1)/Nitric Oxide Synthase (NOS) balance system, which is one of the most important systems in the regulation of the blood flow in several organs, in the placenta and in the decidua in normal and pre-eclamptic women. Since cyclosporine (CsA) has the potential to affect fetal growth and maternal state, through the modulation of ET-1 and NO synthesis, we compared these data with those obtained from transplanted women. Eleven CsA-treated female kidney transplant recipients (Tx), without fetal growth restriction and/or PE, and 12 PE, non transplanted, women were recruited. Fourteen normal pregnancies served as controls. Placental expression of ET-1 was evaluated by in situ hybridization, NO synthase (NOS) by NADPH-diaphorase staining and in situ hybridization. Both PE and Tx showed a marked reduction of NADPH-diaphorase staining, as well as of endothelial constitutive NOS (ecNOS) mRNA, in the syncytiotrophoblast layer of all villi, whereas the expression of inducible NOS was unchanged. Normal placenta showed a strong positive signal of ET-1 along the endothelium of utero-placental arteries within the basal plate which was markedly increased only in the decidua of Tx. Thus, Tx patients showed the mean relevant alteration of ET-1/ecNOS balance, in spite of the lack of any alteration of fetal growth and maternal renal function. We further explored the state of activation of placental endothelial cells, evaluating Tissue Factor (TF) gene expression. TF was strikingly increased only in PE patients, where it is localized in the endothelial cells, as well as in the cytotrophoblast invading the basal decidua. The modification of ET-1/ecNOS vasoactive balance, as elicited by CsA, is unable to account for the major features of the preeclamptic state. In the same patients we compared the preceding results with the uterine and umbilical Doppler velocimetry. We found that all Tx patients showed normal umbilical artery blood flow velocimetry, all but one displayed a normal pattern of uterine artery waveforms. Our studies witness that the ET-1/NOS balance is unable to lead to the modifications of Doppler impedance in the umbilical and uterine arteries.

Introduction

Beside other multiple functions (metabolic, endocrine, hemopoietic, immunological, etc.) of the placenta, the "physiological exchange" [1] of gases and nutrients between foetus and mother is considered the first and fundamental for the growth and the survival-own of the foetus. The placental exchange is a complex function influenced by many different conditions, not only limited to chorionic villi, as frequently thought. On the maternal side, the exchange is conditioned by the general status of the mother (high altitude, nutrition, anemia, systemic diseases) and by the status of utero-placental vessels (maternal blood flow). On the foetal side, the general conditions, particularly of the cardiovascular system, and the status of umbilical vessels (foetal blood flow) have a strong importance. Maternal flow is the result of angiogenesis and morpho-functional transformation of endometrial vessels: both in humans and in other mammalians, maternal angiogenesis is prevalent in early phase of pregnancy and may also compromise the phenomenon of placentation. Foetal flow depends chiefly by chorionic angiogenesis which is very important in the late phase of pregnancy (during the last trimester in humans) [2].

At the placental level the direct exchange is due to the villar and capillary conformation. The enhancement of the materno-foetal in the third trimester depends on three phenomena: a) villar tree branching, b) villar maturation (i.e. formation of vasculo-syncytial membranes), c) development of villar vascular network. These three phenomena are independent, because are determined by different stimuli, and that explains the aspects observed in different placental conditions.

A lot of different factors and their receptors regulate all the phases of maternal and foetal angiogenesis as well as the maturation and the function of villar tree and capillaries, with often a balance mechanism of agonist and antagonist effects. Reynolds & Redmer [3] think that the most important factors regulating angiogenesis are represented by Vascular Endothelial Growth Factor (VEGF), Fibroblast Growth Factors (FGF) and their receptors and Angiopoietins and their receptors. These families of factors are variably expressed by both maternal and placental tissues during the different phases of pregnancy and they are also important in the flow regulation and in the differentiation of several foetal tissues. In particular, the VEGF and the bFGF are implicated in the production of Nitric Oxyde (NO) both in endometrium and in the chorionic villi, with a cyclic and paracrine mechanism, mediated by steroid hormones and their receptors. The action of VEGF is considered to be correlated with the function of the Placental Growth Factor (PlGF) in the regulation of branching/non branching angiogenesis of chorionic villi [4].
The vasoactive functions are performed in particular by the prostacylcin/thromboxane balance [5] and especially by the Endothelin-1 (ET-1)/NO Synthase (NOS) balance. This latter is implied in several vascular disease of the placenta and in the pre-eclampsia: the accumulation of peroxynitrite ONOO(-), a derivate of the oxidative degradation of NO, is considered the responsible of the major dysfunctions of the pre-eclampsia: blood pressure, renal changes, and neural defect [6]. The study of ET-1/NOS balance is considered today of primary importance in the evaluation of placental vascular defects.

Material and Methods

Our studies concerned 14 pregnant women at term, used as control. Eleven kidney transplant women became pregnant with a median of 7.2 years after transplantation and they were treated with a mean dose of 3 mg/Kg of Cyclosporine A (CsA) during pregnancy. Fourteen pre-eclamptic women with foetal growth restriction (FGR) were recruited and submitted to Doppler flow velocimetry of uterine and umbilical arteries.
In all women, placental and basal plate samples were obtained at caesarian section and snap frozen in liquid nitrogen and stored.
For the evaluation of the presence of NOS, staining with the reduced form of Nicotinamide-Adenine Dinucleotide Phosphate (NADPH)-diaphorase was performed on frozen sections according to Conrad et al. [7].
For the evaluation of NOS isoforms, ET-1 and Tissue Factor (TF), the RNA probes were obtained using the appropriate complementary DNA fragments and subcloning them with plasmides as described [8]. Prehybridization, hybridization, removal of non specifically bound probe and other procedures were previously described [9].
The optical density of signal generated by silver grains (in situ hybridization) and nitroblue tetrazolium crystals (NADPH staining) was automatically quantified. The video image generated by a video camera connected to a Leica microscope was equipped with a Power PC computer with a frame grabber. Single images were digitized for image analysis at 256 gray levels. An optical threshold and filter combination was set to select only silver grains or nitroblue tetrazolium crystals.

Results

The newborn's weight of Tx patients was appropriate for gestational age (mean 2,467.27 g for 34.45 weeks) while in the PE women the weight was significantly lower (1,488.18 g for 31.4 weeks) as respect to normal women (3,432 g for 38.07 weeks).
In all PE patients artery blood flow velocimetry resulted abnormal. Umbilical artery showed an abnormal Doppler pattern in all but one PE patients: particularly, 5 PE women showed absent and 1 reverse end-diastolic flow. Two perinatal deaths occurred in this latter group. All Tx patients showed normal umbilical artery blood flow velocimetry, all but one displayed a normal pattern of uterine artery waveforms. In all women with normal pregnancies both uterine artery and umbilical artery blood flow velocimetry were normal, as expected.
The NADPH-diaphorase staining was localized mainly in the syncythiotrophoblast layer of normal stem, intermediate and terminal villi and was markedly reduced in placentas both of Tx and PE patients. The reaction was always absent in cytotrophoblast cells and in the villar endothelium.
In normal villi, ecNOS mRNA showed the same pattern of distribution of NADPH-diaphorase, with a more intense signal in the syncythium of terminal villi. The endothelium of stem villous vessels was also positive. In situ hybridisation signal for ecNOS mRNA in the syncythial layer was significantly reduced both in Tx and in PE patients: quantitative value was $195\pm20 \times 10^3$ in normal women; $87.7\pm19.3 \times 10^3$ in Tx; $95\pm12 \times 10^3$ in PE ($p<0.01$ for both pathological groups). In the basal plate the ecNOS was expressed in large cells of extravillar trophoblast without differences in the different groups.
The iNOS mRNA was absent in the chorionic villi and it was expressed in the deciduas. No differences were observed in normal and pathologic pregnancies.
Normal placenta showed a strong positive signal of the vasocostrictor ET-1 along the endothelium of stem villous vessels and of utero-placental arteries within the basal plate. The pattern of tissue distribution and signal intensity remained unchanged in PE placentas, whereas it was markedly increased in decidua of transplant recipients.
Differences in the ecNOS and ET-1 gene expression observed in Tx, with respect to normal women, were correlated with the different levels of exposure to the CsA therapy (? corrected for ties, -0.674 for ecNOS and 0.644 for ET-1; both $p<0.01$).
Placentas of healthy pregnant women and those of Tx patients showed a very faint TF signal in placental endothelial and perivascular cells. On the contrary, TF turned out to be strikingly expressed in PE women in

endothelial cells as well as in cytotrophoblasts invading the basal deciduas: normal 2.95±4 x 10^3 pixels; PE 14.6±9 x 10^3 pixels).

Discussion

In placental tissue the CsA also confirms its capacity to induce the synthesis of ET-1 and to inhibit the NO release, as observed in other human organs, with a possible occurrence of hypertension and nephrotoxicity in chronically treated transplant patients [10,11]. However, in our experience this effect, clearly dependent on the immunosuppressive dose level, is not correlated with hypertension, renal damage and fetal growth restriction. We stressed that, whereas the effects of CsA seem to be similar to those observed in PE women, the expression of TF is not modified in Tx women and on the contrary increased in PE ones. We can suppose that immunosuppressive therapy inhibits cytokine and growth factor synthesis and release, including tumor necrosis factor-? and other nuclear factors, thus interfering with TF gene induction. The conclusion is that the NOS/ET-1 system is unable *per se* to interfere with the placental exchanges and the foetal growth, and the action of CsA on foeto-placental vessels could be related to other vasoactive factors as prostacyclin/thromboxan system, not considered in our study.

The complexity of the placental angiogenesis and vascular tone are also proved by the studies of the NOS/ET-1 balance system in PE women. We observed a clear decrease of ecNOS and an increase of ET-1 in placenta and in maternal decidua, as observed by some Authors, according to the abnormal Doppler of uterine arteries, which records the decrease of maternal blood flow [12,13,14]. Other Authors, nevertheless, found a different distribution and behaviour of ecNOS, iNOS, and ET-1, in a way in strong contrast with these experiences [15,16,17]. By a general point of view, the reasons of this phenomenon may be different. The experimental models compare the human disease of pre-eclampsia and artificial observations in animals (the pre-eclampsia is not naturally present out of the humans). The methods used for the studies is very different (immunohistochemistry, in situ hybridization, research in cell coltures, extraction of substances and/or vasoactive factors) and not easily comparable.

However we can suppose that the most important reason is referable to some distinct and different pathogenetic models in PE. The generally accepted assumption that foetal hypoxia of PE pregnancy with foetal growth restriction invariably coexists with placental hypoxia has been recently challenged: foeto-placental hypoxia may arise from either restricted oxygen entry into utero-placental tissue or, alternatively, from inadequate gas transfer from the intervillous space to the foetus. Consequently, placental villi at delivery may have been recently exposed to hypoxia or "hyperoxia". On the basis of what observed in the idiopathic foetal growth restriction [18,19] and in our observation on umbilical artery Doppler in PE condition, we can suppose two different types of vascular pathogenesis in PE.

The type I, more frequent, is correlated with a condition of utero-placental hypoxia. The amount of oxygen transferred from the maternal blood to the placenta is decreased, the prastacyclin/thromboxan system is modified, the VEGF is increased, the NO is enhanced, the trophoblast turn over is favoured, the angiogenesis is stimulated.

The type II is correlated with a condition of relative placental "hyperoxia". The maternal flow is less or precociously decreased, the VEGF/PIGF balance system is modified with an increase of PIGF production, the villous angiogenesis is reduced and a condition of relative hyperoxia of the intervillar spaces with respect to foetal blood is evident, the production of NO is inhibited and the foetal end diastolic flow is decreased or inverted.

The different pathogenetic mechanism could explain not only the contrasts in biological experiences, but also in different aspects known in the morphology of pre-eclamptic placentas. In any case, it is evident that each study on foetal and maternal angiogenesis and vascular modulation in pregnancy is only limited to a single particular aspect of the complex phenomenon of the maternal-foetal exchanges, and for whose comprehension several different approaches are necessary.

Key words

Human placenta, nitric oxide sinthase, endothelin-1, tissue factor.

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