Increase in the relative level of type V collagen in the placentae of patients with pre-eclampsia

Masaaki Iwahashi¹, Akira Ooshima² and Ryosuke Nakano¹,³

¹Department of Obstetrics and Gynaecology, and ²Department of Pathology, Wakayama Medical College, 27 Shichibancho, Wakayama 640, Japan
³To whom correspondence should be addressed

To obtain some insight into the extracellular matrix in the human placenta, we investigated the composition of collagens purified from the placentae of patients with pre-eclampsia and compared it with normal placentae. Collagen was extracted from the placentae of both normal and pre-eclampsia pregnancies during the third trimester. The relative amounts of various collagens were evaluated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis. The ratio of the intensity of the band corresponding to the α1(I) chain with that of the α1(III) chain in placentae of pre-eclampsia was significantly lower than in normal placentae (P < 0.05). In contrast, the ratio of the intensity of the band corresponding to the α1(V) chain with that of the α1(I) chain in placentae of pre-eclampsia was significantly higher than in normal placentae (P < 0.05). The results suggest that an increased level of type V collagen relative to type I collagen in the placentae of pre-eclampsia might be closely associated with the disturbance to trophoblastic cell functions and the supply of nutrients to the developing fetus necessary for the maintenance of pregnancy.

Key words: placenta/pre-eclampsia/type V collagen

Introduction

Pre-eclampsia is an important complication of pregnancy, and a major cause of maternal or fetal morbidity and mortality. Salafia et al. (1995) suggested that the placental pathological features of preterm pre-eclampsia might include uteroplacental vascular lesions and placental lesions such as villous fibrosis, hypovascularity, increased syncytiotrophoblast knotting, villous infarct, and abruptio placentae. However, little is known about the change in the composition of the extracellular matrix (ECM) in the human placenta during pre-eclampsia.

The ECM is considered to play an pivotal role in the stability of tissue structure and in the regulation of cell growth and differentiation (Madri and Basson, 1992; Lin and Bissell, 1993). The distribution of components of the ECM, such as various collagens, fibronectin, and laminin, in the human placenta has been studied by immunohistochemical methods (Earl et al., 1990; Blankenship and King, 1993; Nanaev et al., 1995). Among the various collagens, type V collagen was originally described as a component of chorionic and amniotic membranes (Burgeson et al., 1976). It is thought to play a major role in maintaining a barrier against pathogens and inflammatory cells, and in preventing the loss of amniotic fluid (MODESTI et al., 1984). In the present study, we attempted to investigate the levels of type V collagen relative to the levels of type I collagen in the extracts of placent al tissues during pre-eclampsia in the third trimester of pregnancy.

Materials and methods

This project was approved by the Committee on Investigations Involving Human Subjects of Wakayama Medical College. Informed consent was obtained from each subject after the purpose and nature of the study had been fully explained.

Tissues

Normal placentae (36–38 weeks gestation, n = 21), obtained at vaginal delivery from women aged 19–39 years with uncomplicated pregnancies, were taken for investigation. Placentae (32–36 weeks gestation, n = 11), were also obtained at Caesarean section from women with pre-eclampsia; the clinical data are summarized in Table I. Chorionic and amnionic tissue was excluded, together with necrotic, infarcted and haemorrhagic areas. Pieces were cut from three separate central zones of the placenta.

Sample preparation

Minced samples of human placenta (10g wet weight) were washed overnight in cold distilled water and freed of blood. Tissues were homogenized in 50 volumes of 0.5 M acetic acid containing 1 mg/ml pepsin (Sigma Chemical Co., St. Louis, MO, USA). Collagens were extracted using previously described methods (Sykes et al., 1976). The solubility of the tissue collagen from each placent al sample was estimated by comparing the hydroxyproline content of the initial homogenate with that of the final solution of collagen (Kivirikko and Prockop, 1967). As a standard, Type V collagen was isolated by salt precipitation from pepsin digests of human placental tissues as described elsewhere (Furuto and Miller, 1980; Miller and Rhodes, 1982). The extracted type V collagen was lyophilized.

Electrophoresis

The relative abundance of the α1(III) chain and the α1(V) chain were estimated by interrupted gel electrophoresis, as described by Sykes et al. (1976). Electrophoresis was carried out in an 8% polyacrylamide gel slab (Sigma), as described by Laemmli (1970). Lyophilized samples of placental collagens and type V collagen
(0.2 mg/ml) were redissolved and denatured by heating in gel buffer containing 1% SDS at 60°C for 30 min. Aliquots of 25 µl of denatured collagens and 5 µl of denatured type V collagen as standard were applied to the gel and subjected to electrophoresis at 80 mA for 1.5 h. Sample wells were then filled with a 20% solution of β-mercaptoethanol (Wako Chemical Co., Osaka, Japan) to cleave intramolecular disulphide bonds of type III collagen, [α1(III)]. Electrophoresis was then continued for another 1 h. After staining of the gel with Coomassie Brilliant Blue (Sigma Chemical Co.) the intensities of the bands were quantified by densitometry. The relative amounts of α1(III) or α1(V) chains were calculated by dividing the areas under the densitometric peaks for the bands corresponding to α1(III) and α1(V) by that corresponding with α1(I).

**Statistical analysis**

The ratio of α1(III) to α1(I) chains and that of α1(V) to α1(I) chains, as estimated by densitometry, are presented as means ± SEM. Results were analysed by analysis of variance and unpaired t-tests.

**Results**

Although the levels of α1(I) chains were similar in the placentae obtained from normal pregnancy and pre-eclampsia, those of α1(V) chains were increased and those of α1(III) chains were decreased in the placenta of pre-eclampsia compared with those of normal pregnancy (Figure 1). The data are summarized in Table II. The mean ratio of the intensity of the band of α1(III) to that of α1(I) in placenta of normal pregnancy was significantly lower than that in placenta of pre-eclampsia (P <0.05). In contrast, the mean ratio of the intensity of the band of α1(V) to that of α1(I) in placenta of pre-eclampsia was significantly higher than that in placentae of normal pregnancy (P < 0.05).

**Discussion**

In the present study, we investigated changes in the composition of collagens in the placentae of normal and pre-eclampsia pregnancies. We were able to solubilize 70-85% of the collagen present in the human placental tissues, as measured by reference to levels of hydroxyproline (data not shown). Therefore, we postulated that the extracted collagen might accurately reflect the entire complement of collagen in the sample tissues.

Pre-eclampsia is defined by hypertension and proteinuria or generalized oedema developing after 20 weeks gestation. It develops into eclampsia when accompanied by a maternal seizure. Despite extensive research, the aetiology of pre-eclampsia remains unknown. The placental pathology associated with pre-eclampsia might include various vasculopathy such as acute atherosclerosis and acute atheromatous changes (Van der Veen et al., 1982; Las et al., 1985).

Although type I and type III collagens are commonly found in combination, the ratios of type III to type I collagen in the placentae in pre-eclampsia were significantly lower than those in normal pregnancy. The most striking finding was a significant increase in the relative amount of type V collagen in the
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placentae of pre-eclampsia as compared with normal pregnancy. Changes in the ratio of type III to type I collagen have been demonstrated in the human skin with ageing (Sykes et al., 1976) and during the development of human atherosclerosis (McCullagh and Balian, 1975; Ooshima, 1981). Increased relative amounts of type V collagen have been reported in atherosclerotic tissue (Van der Veen et al., 1982). In a previous report, we found that the ratio of type III to type I collagen was lower in term placentae than in premature placentae. In contrast, the ratio of type V to type I collagen was significantly higher in term than in premature placentae. This suggested a change in these ratios in the placenta during the process of development and ageing (Iwahashi et al., 1996). In contrast, the ratio of type III to type I was lower, and that of type V to type I was higher, in placentae from pre-eclampsia compared with normal term placentae.

In this respect, it is suggested that the changes of ECM in the placenta of pre-eclampsia might be similar to those of atherosclerosis or advanced ageing. The possible cause of changes in the composition of the ECM in the placenta of pre-eclampsia might involve exposure to hypertension during pregnancy, and an alteration in the density of cells in the human placentae. Recently, cell density-dependent effects have been reported in a number of types of cell, such as mesangial cells (Ishimura et al., 1989; Lermioglu et al., 1991; Worthuis et al., 1993), vascular smooth muscle cells (Goodman and Majack, 1989; Majors and Ehrhart, 1992), and fibroblasts (Halme et al., 1986; Rösner et al., 1990). It has been suggested that cell density might modulate biological behaviour, causing changes in signal transduction responses to hormonal stimulation, in the growth, synthesis and composition of the ECM, and in the synthesis of specific proteins (Lermioglu et al., 1991; Worthuis et al., 1993). Worthuis et al. (1993) reported that mesangial cells synthesized relatively more type I collagen per cell at higher cell densities, whereas rates of synthesis of type III and type IV collagens per cell did not depend on cell density.

The distribution of type V collagen in placentae has been determined by immunohistochemical staining (Nanaev et al., 1993). Changes in the relative amount of this collagen in the placenta of pre-eclampsia have not been fully clarified. Purified type V collagen, extracted from placentae by differential salt precipitation, was composed of α1(V), α2(V) and α3(V) chains, with α1(V) being predominant (Figure 1). This finding is consistent with previously reported data (Christopher et al., 1984). Therefore, the relative levels of α1(V) were calculated in terms of α1(f). Thus, it seems that placental stromal cells in preeclampsia, which are the major collagen-producing cells, synthesize type V and type I collagens predominantly with a lower amount of type III collagen. Type V collagen has the ability to bind to insulin (Yaoi et al., 1991) and to heparin/heparan sulphate (Richard et al., 1989). Other studies have indicated that insulin bound to type V collagen retains mitogenic activity (Yaoi et al., 1991) and that heparin/heparan sulphate modulates the biological activities of vascular endothelial cell growth factor (Lobb et al., 1986) and basic fibroblast growth factor (Thornton et al., 1983; Schreiber et al., 1985). These findings suggest that type V collagen and the presence of bound factors might play important roles in the development and causes of pre-eclampsia. Further studies have suggested that hyperinsulinaemia might be associated with hypertension in pregnancy or pre-eclampsia (Singh, 1976; Bauman et al., 1988; Solomon et al., 1994; Fuh et al., 1995). Therefore, it is suggested that increased relative levels of α1(V) or type V collagen might result in increased amounts of insulin molecules in the placenta of pre-eclampsia. This hypothesis might provide a biochemical basis for the functional role of the placenta.

In conclusion, the human placenta of pre-eclampsia is characterized by markedly increased relative levels of type V and type I collagens. The results suggest that alterations in the composition of collagen in the placenta of pre-eclampsia might play an important role in the disturbance of trophoblastic cell functions and in the supply of nutrients to the developing fetus necessary for the maintenance of pregnancy.

References


Halme, T., Vihersaari, R. and Penttinen, R. (1986) Lysyl oxidase activity and the distribution of type V collagen in placentae has been suggested that increased relative levels of type V collagen in the placenta of pre-eclampsia might be similar to those of atherosclerosis or advanced ageing. The possible cause of changes in the composition of the ECM in the placenta of pre-eclampsia might involve exposure to hypertension during pregnancy, and an alteration in the density of cells in the human placentae. Recently, cell density-dependent effects have been reported in a number of types of cell, such as mesangial cells (Ishimura et al., 1989; Lermioglu et al., 1991; Worthuis et al., 1993), vascular smooth muscle cells (Goodman and Majack, 1989; Majors and Ehrhart, 1992), and fibroblasts (Halme et al., 1986; Rösner et al., 1990). It has been suggested that cell density might modulate biological behaviour, causing changes in signal transduction responses to hormonal stimulation, in the growth, synthesis and composition of the ECM, and in the synthesis of specific proteins (Lermioglu et al., 1991; Worthuis et al., 1993). Worthuis et al. (1993) reported that mesangial cells synthesized relatively more type I collagen per cell at higher cell densities, whereas rates of synthesis of type III and type IV collagens per cell did not depend on cell density.

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