Effect of first-trimester serum from pregnant women with high-resistance uterine artery Doppler resistance on extravillous trophoblast invasion

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BACKGROUND: Abnormal uterine artery Doppler indices are associated with pregnancy complications such as pre-eclampsia and intrauterine growth restriction. Poor trophoblast invasion may be a consequence of, or be associated with, abnormal Doppler indices. OBJECTIVE: To evaluate in vitro trophoblast function following exposure to first-trimester serum from pregnancies with high uterine artery Doppler resistance indices. METHODS: Doppler ultrasound examination of the maternal uterine arteries was performed on women at 10–14 weeks’ gestation. Serum was collected from women with bilateral uterine artery notches with resistance indices above the 95th centile and from patients with normal uterine artery indices. The effect of serum on trophoblast invasion was determined using an established in vitro model from the extravillous trophoblast-derived cell line SGHPL-4. RESULTS: Trophoblastic invasion was significantly reduced when treated with serum from women with high-resistance compared with normal-resistance uterine artery Doppler indices (P < 0.05). CONCLUSION: Maternal serum in the first trimester of pregnancy from patients with high-resistance uterine artery Doppler indices appears to inhibit trophoblast invasion. This experimental model allows further investigation of factors responsible and the evaluation of therapeutic strategies.

Key words: Doppler/pregnancy serum/trophoblast invasion/uterine artery resistance

Introduction

Trophoblast invasion and regulation is integral to a successful pregnancy. Extravillous trophoblast (EVT) invasion leads to replacement of the endothelium of the uterine spiral arteries. This process modifies the high-resistance, nonpregnant uterine circulation to a low-resistance system in order to facilitate exchange of nutrients and waste with the fetus (Sadler, 2000). Normal placental development is determined by the strict temporal and spatial regulation of trophoblast invasion and apoptosis (Halperin et al., 2000; Thiet et al., 2000). The precise factors responsible for this regulation in vivo are unknown but are thought to be autocrine and/or paracrine in nature (Bischof et al., 2000).

Failure of normal trophoblastic invasion is thought to lead to the persistence of a high-resistance uteroplacental circulation that can be detected by uterine artery Doppler ultrasound from as early as 11 weeks’ gestation (Harrington et al., 1997; Prefumo et al., 2004a). Inadequate trophoblast invasion is associated with conditions such as fetal growth restriction and pre-eclampsia (Meekins et al., 1994; Kingdom, 1999). If this hypothesis on the aetiology of pre-eclampsia is correct, any causative humoral factor is likely to be produced in early pregnancy and be present in higher concentrations in women with abnormal, high-resistance uterine Doppler indices. The aim of this study was to investigate the effects of first-trimester serum from women with normal- and high-resistance uterine artery Doppler indices on EVT invasion.

Materials and methods

This was a prospective study carried out on women attending a pregnancy dating clinic prior to termination of pregnancy. Women who gave written consent to take part in the study had blood drawn and underwent Doppler ultrasound examination of the uterine arteries. Only singleton pregnancies were included. Women with a known medical condition or a history of recurrent miscarriage were excluded. Gestational age was calculated from the last menstrual period and confirmed by crown–rump length measurement. Examinations were performed using an Acuson XP-10 system (Mountain View, CA, USA) equipped with a 5-MHz curvilinear transabdominal probe, following the technique previously described (Harrington et al., 1997). The resistance index (RI) was measured and recorded, as well as the presence or absence of an early diastolic notch. Patients with high and normal uterine artery Doppler were identified, and their serum was collected. High-resistance cases were defined as those presenting with bilateral uterine artery notches and a mean RI above the 95th centile.
(Hollis et al., 2003). Samples were also obtained from patients with normal Doppler indices (no uterine artery notches and a mean RI below the 95th centile).

The serum was stored at –20°C and thawed at room temperature just before use. The human EVT cell line SGHPL-4, derived from primary EVT cells, was used throughout this study (Choy and Manyonda, 1998). SGHPL-4 cells retain many features of normal EVT, such as expression of cytokeratin-7, BC-1, HLA-G, CD9, hPL, and HCG (Choy and Manyonda, 1998; Cartwright et al., 1999; Shiverick et al., 2001; Prefumo et al., 2004b). SGHPL-4 cells have been used to study trophoblast invasion in vitro and behave in the same manner as primary cells.

Invasion assays were carried out using a modification of a previously described method (Cartwright et al., 1999). In brief, SGHPL-4 cells were incubated with gelatin-coated microcarrier beads to allow the cells to adhere to the beads. Individual bead–cell complexes were suspended in fibrinogen, and clotting was promoted using thrombin. Once set, Ham’s F-10 medium without serum was added. Serum samples collected from pregnancies with high and normal uterine artery resistance were heat inactivated at 56°C for 30 min. Serum (10% v/v) samples were then added to gels and incubated for 96 h. Images of 20 beads per plate were taken at random. The researcher was blinded to the type of serum in each plate until the analysis was completed. The beads were visualized using an Olympus (Middlesex, UK) IX50 inverted microscope at ×60 magnification, and images were captured using a digital camera (JVC TK-C1360E CCD). For each bead, the length of the cellular processes formed was measured and given an arbitrary length, using Image Pro-Plus software (Media-Cybernetics, Silver Spring, MD, USA). Invasion was determined as any process greater than the average radius of a bead. All experiments were performed in duplicate and repeated three times with different patients’ serum samples.

Statistical analysis

Comparisons of the demographic characteristics between groups were carried out using Fisher’s test. Using Mann–Whitney U-test and comparison of proportions tests, the length of the SGHPL-4 cell processes formed was analyzed for significant differences between cases and controls.

Results

Serum was obtained from seven women with high and from seven women with normal uterine artery Doppler resistance indices. Comparison of demographic data between the cases and controls is shown in Table I. There were no significant differences in maternal age, ethnicity and gestation of sampling. As expected, the mean RI in the high-resistance group (0.87) was significantly different (P < 0.001) from that in the control group (0.63).

<table>
<thead>
<tr>
<th>Table I. The mean (range) maternal age, uterine artery Doppler resistance index (RI), gravidity, parity and racial origin of the women in the high- and normal-resistance uterine artery Doppler groups</th>
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<tbody>
<tr>
<td>High RI</td>
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<tr>
<td>Maternal age</td>
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<tr>
<td>Uterine artery RI</td>
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<td>Gravidity</td>
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<td>Parity</td>
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<td>Percentage Caucasian</td>
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NS, not significant. There were no significant differences in any of the demographic features except for mean RI.

The development of finger-like processes from the EVT-derived cell line SGHPL-4 occurred after 24 h in culture. The invasion of the fibrin gels by SGHPL-4 cells was determined by measuring the length of these cellular processes after 4 days using digital image microscopy (Figure 1). The number of measured invasive processes was 288 for the control, 322 for the normal-resistance group and 357 for the high-resistance group. The degree of cell invasion was significantly greater with serum from women with normal- compared with women with high-resistance uterine artery Doppler resistance indices (P < 0.05) (Figure 2).

Discussion

Regulation of trophoblast invasion in early pregnancy is essential for normal placental development. The data of this study demonstrate that serum from women with high-resistance first-trimester uterine artery Doppler indices, which is indicative of poor trophoblastic invasion, is associated with significantly reduced trophoblast invasion when compared with normal pregnancy serum.

Doppler ultrasound investigation of the uterine arteries has been used for many years as a noninvasive technique to assess uterine vascular resistance and, indirectly, the progress of the physiological transformation of the decidual and myometrial vessels. The association between uterine artery resistance indices and the degree of trophoblast development has been demonstrated
Figure 2. Effect of serum from women with high and normal uterine artery Doppler resistance indices on SGHPL-4 cell invasion. Fibrin gels containing SGHPL-4-coated beads were incubated for 96 h in the presence of serum from women with high and normal uterine artery Doppler resistance indices in the first trimester. Invasive processes were measured following random selection of 20 beads for each treatment. Experiments were performed in triplicate, and the results are presented as mean (+SEM) of three pooled experiments (*P < 0.05).

on histological studies of placental development (Lin et al., 1995; Aardema et al., 2001; Prefumo et al., 2004b). Additionally, not only is a high-resistance Doppler pattern in the first trimester associated with a higher incidence of intrauterine growth restriction (Van den Elzen et al., 1993; Harrington et al., 1997; Martin et al., 2001; Vainio et al., 2002) but also abruptio, intrauterine death and pre-eclampsia (Hollis et al., 2003; Conde-Agudelo et al., 2004). The latest consensus statement from the World Health Organization (WHO) concludes that uterine artery Doppler is the best, currently available screening test for assessing the risk of a pregnancy being complicated by the development of pre-eclampsia (Conde-Agudelo et al., 2004).

In the aetiology of pre-eclampsia, a high-resistance uterine artery circulation is thought to cause chronic placental underperfusion. The underperfusion of the placenta results in the release of humoral, pre-eclamptic factors into the maternal circulation that cause endothelial dysfunction, leading to the development of hypertension and proteinuria (Myers and Baker, 2002). Although abnormal uterine artery Doppler has been demonstrably associated with measured changes in many humoral factors capable of producing endothelial dysfunction in the mother (Holden et al., 1998; Dash et al., 2003), these studies have all been conducted in later pregnancy or after the development of pre-eclampsia. There are no data on the effects of serum from women with abnormal uterine artery Doppler indices in the first trimester on trophoblast function. The results of this study show that serum from pregnant women in the first trimester can stimulate EVT invasion and that this response is attenuated if serum from women with high-resistance Doppler indices is used. As serum samples were obtained from women undergoing termination of pregnancy, outcome data relating to the complications of pre-eclampsia and fetal growth restriction were not possible.

Conclusion

Trophoblast invasion in early pregnancy is modulated by balancing factors that promote and inhibit invasion within the uterine environment (Clark et al., 1996; Williams et al., 1997). Our previous studies have demonstrated that poor trophoblastic invasion and modification of uterine spiral arteries are associated with abnormally high uterine artery Doppler resistance indices (Prefumo et al., 2004b). In this study, we have tested the hypothesis that maternal serum from patients with high-resistance uterine artery Doppler indices may modulate trophoblast invasion. Although the sample size was small, our results indicate that maternal serum from the first trimester contains factors that regulate trophoblast invasion and that serum from pregnancies with high uterine artery resistance is less able to support this process. Further study of serum from these patients in early pregnancy is required to identify the factors that may be contributing to reduced invasion. Our model may therefore be used to investigate the factors responsible for the modulation of trophoblast function and perhaps eventually lead to the development of new therapeutic strategies or a more accurate noninvasive screening test for pre-eclampsia.

References


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