The role of insulin-like growth factor binding protein-1 phosphoisoforms in pregnancies with impaired placental function identified by Doppler ultrasound

D.Fowler1,2, G.Albaiges1, C.Lees1, J.Jones2, K.Nicolaides1 and J.Miell3,4

1Harris Birthright Research Centre for Fetal Medicine, Department of Obstetrics & Gynaecology, King’s College Hospital, Denmark Hill, London SE5 9RS and 2Department of Medicine, Guy’s, King’s College & St Thomas’ Medical School, Bessemer Road, London SE5 9PJ, UK
3Present address: Genetics Unit, Massachusetts General Hospital, 55 Fruit Street, Warren 801, Boston, MA 02114-2696, USA
4To whom correspondence should be addressed

This study was performed to investigate the hypothesis that insulin-like growth factor binding protein-1 (IGFBP-1) is involved in the pathogenesis of trophoblast invasion and impaired placentation in human pregnancy. The role of total and non-phosphorylated IGFBP-1 in women with fetal growth restriction and in high risk pregnancies identified by uterine artery Doppler ultrasound screening was examined. This was a prospective study of women booked for antenatal care having second trimester anomaly scans and Doppler screening between 22-26 weeks gestation. Women were divided into three groups and compared: normal uterine artery Doppler and normal fetal growth (control group, n = 10); abnormal Doppler and normal fetal growth [bilateral uterine artery notches (BN; n = 16); abnormal Doppler and intrauterine growth restriction (IUGR; n = 8)]. Maternal serum was collected, stored and assayed simultaneously for total and non-phosphorylated IGFBP-1. There was elevated total and non-phosphorylated IGFBP-1 (mean 44.99 ± 12.19 and 29.61 ± 10.38 ng/l respectively) in the IUGR group compared with controls (mean 17.96 ± 3.24 and 12.18 ± 1.55 ng/l, P < 0.05). This finding suggests that the various IGFBP-1 isoforms, the degree of phosphorylation and the ratios of these different forms locally may be important during trophoblast invasion and may be implicated in clinical manifestations of impaired placentation later in the second trimester.

Key words: Doppler ultrasound/insulin-like growth factor binding protein-1/intrauterine growth restriction/trophoblast invasion

Introduction

Fetal growth is determined by many disparate influences, among these are genetic factors, the intrauterine environment and uteroplacental blood flow. It is these factors and the complex interactions between mother, placenta and fetus which make the study of intrauterine growth so difficult. Recently several workers have shown that genetic polymorphisms in the glucokinase and insulin genes (INS VNTR) are important in the determination of birthweight; by inference these factors are likely to be important in intrauterine growth (Dunger et al., 1998). Other molecules structurally related to insulin may also be important in fetal growth and placental physiology.

The insulin-like growth factors (IGF) and their binding proteins are known to be important in the human female menstrual cycle and in pregnancy (Zhou et al., 1994; Han et al., 1996). Insulin-like growth factor binding protein-1 (IGFBP-1) was the first member of a family of structurally related soluble proteins to be characterized (Lee et al., 1988). This binding protein family is involved in modulating the effects of IGF-I and -II which are important in growth, metabolism and development. IGFBP-1 is an endocrine factor altering serum IGF bioavailability, important in both maternal and fetal physiology (Reece et al., 1994). IGFBP-1 also acts in a paracrine/autocrine way in the endometrium, important in the menstrual cycle, implantation and trophoblast invasion. It is unclear what the involvement of the IGF/IGFBP system is in the maintenance of placentation function. The exact biological function of endometrial secretory proteins in primates, including the two most characterized, glycodelin (PP14) and IGFBP-1, however, remains to be elucidated. Glycodelin is thought to have immunosuppressive qualities and IGFBP-1 may regulate trophoblast migration. In the baboon IGFBP-1 is observed in the deep basal glands during the luteal phase (Fazleabas et al., 1997). In human endometrium mRNA species encoding IGFBP-1–6 are expressed, mainly in stroma, and are expressed differentially in the progesterone secretory phase with IGFBP-1 being the predominant species (Han et al., 1996). In-vitro cell culture work supports this, in particular the 1.5 kb mRNA transcript of IGFBP-1 appears to be detected only in progestagen-treated cells. IGFBP-1 mRNA in human secretory endometrial stromal cells appears to be under hormonal influence at the transcription rather than the translation level (Liu et al., 1997). Trophoblast is also subject to several regulators. Proliferation of a normal placental cytotrophoblast cell line (NPC) showed that epidermal growth factor (EGF), transforming growth factor alpha (TGF-α) and IGF-1 stimulated NPC cell proliferation. In contrast, transforming growth factor β-1 (TGF-β-1) was found to be a negative regulator, inhibiting EGF-induced cell proliferation (Li and Zhuang, 1997).

The physiological role of IGFBP-1 may depend on differential phosphorylation of different IGFBP-1 isoforms (Martina et al., 1997). Five IGFBP-1 variants have so far been reported, differing in their phosphorylation state and relative expression in serum, amniotic fluid and decidua. Non-phosphorylated IGFBP-1 has a 4-6-fold lower affinity for IGF compared with phosphorylated IGFBP-1 variants (Jones et al., 1991;
Westwood et al., 1995). Since it has less affinity and less binding for IGF, non-phosphorylated IGFBP-1 would be expected to be less inhibitory and hence allow greater IGF signalling.

The interaction of trophoblast derived IGF-II and decidually derived IGFBP-1 is important in implantation and trophoblast invasion (Irving et al., 1995). Normal trophoblast invasion is physiologically regulated compared with the dysregulation seen in trophoblast tumours. Invasive trophoblast, expressing IGF-II, and decidual IGFBP-1 interact in a highly regulated way (Jones et al., 1993). IGFBP-1 tends to inhibit IGF-II, providing a maternal restraint on invasion. This is important because IGF-II is mitogenic and IGF-II overexpression has been implicated in tumorigenesis.

Previous workers have used endometrial proteins, such as IGFBP-1, as markers of trophoblast invasion, arguing that changes in the maternal vascular export of these decidual proteins reflects reduced trophoblastic invasion and remodelling of the endometrium (Bryant-Greenwood et al., 1993; Zeimet et al., 1993). By inference, several workers have used these decidual proteins as plasma biochemical markers in intrauterine growth retardation (IUGR) and pre-eclampsia (Howell et al., 1989; de Groot et al., 1996) in the hope of developing screening tests.

Intrauterine growth restriction and pre-eclampsia tend to present late in the second/early third trimester. Pathological changes, however, are likely to have occurred much earlier in the pregnancy perhaps because of abnormal trophoblast invasion. Doppler ultrasound of the uteroplacental circulation has been applied as a screening modality in the second trimester for conditions arising from impaired placentaion. It is possible qualitatively to assess uteroplacental flow velocity waveforms by noting the presence of early diastolic "notches" and deriving quantitative measures of impedance such as raised pulsatility (PI) and resistance indices (RI). Abnormal Doppler is characterized by high resistance, bilateral notched uterine artery waveforms which may imply a higher risk for IUGR and pre-eclampsia (Bower et al., 1993; Harrington et al., 1996).

In this study the relationship between total and non-phosphorylated IGFBP-1 and abnormal placentaion in the second trimester of pregnancy was examined. Two high risk subgroups of women with abnormal placentaion were identified: those with high resistance, bilateral uterine artery notches but, at the time of screening, appropriately grown babies and those with similarly abnormal uterine artery Doppler but IUGR babies.

Materials and methods

Women attending the obstetric ultrasound clinic for second trimester scanning between 22 and 26 weeks were recruited to the study, which had research ethics permission. All ultrasound examinations were performed by either G.A. or C.L. using Aecuson Aspen (Acuson Co., Mountainview, CA, USA) ultrasound equipment with 3.5 MHz transabdominal probe and colour Doppler facility. Venous blood was taken from the antecubital fossa at the time of the ultrasound examination (0900–1700), separated within 1 h and serum stored at −20°C. All women were healthy at the time of screening. In particular, all had singleton pregnancies with no obvious fetal abnormalities and none had a prior history of hypertension, diabetes or other cardiovascular disorders or were on any regular medications.

Three groups of women between 22 and 26 weeks gestation were recruited to the study over the same time period: normal uterine artery flow and fetal growth (randomly selected controls, C, n = 10); bilateral notches: normal fetal growth but bilateral uterine artery notches and high resistance indices (mean >0.65) on Doppler examination (BN, n = 16); IUGR: bilateral uterine artery notches and IUGR (n = 8). Pregnancies were identified with IUGR according to ultrasound and Doppler criteria: abdominal circumference <5th centile; abnormal fetal Doppler waveforms (umbilical artery PI >95th centile or absent/reversed end-diastolic flow.

Blood for assay was taken before maternal steroid administration when considering preterm delivery.

Demographic characteristics of the women studied are shown in Table I. This study was undertaken in a UK inner city teaching hospital obstetric ultrasound clinic which is likely to have more high risk pregnancies than a UK district general hospital. This centre serves a multi-ethnic urban population of ~250 000 people living in Brixton, Camberwell, Peckham and Dulwich in the south London Boroughs of Lambeth and Southwark. The IUGR group were all King’s College Hospital patients and were not referred. Workers in this department have been interested in the early diagnosis of IUGR and pre-eclampsia after 22 weeks but before overt clinical presentation. These factors may explain why it was possible to recruit these IUGR cases (n = 8) to the study.

Pregnancies were identified with pre-eclampsia if the blood pressure was 140/90 mmHg on two separate occasions with > 1+ proteinuria on urine reagent sticks or >300 mg protein/24 h on urinary protein collection.

Assays

Maternal serum concentrations of total and non-phosphorylated IGFBP-1 were measured by immunoradiometric assay (IRMA) (DSL TX, USA). The total IGFBP-1 IRMA has a sensitivity of 0.4 μg/l and inter-assay coefficient of variation (CV) of 2.5% at 10.0 and 6.9% at 123 μg/l respectively. The intra-assay CV was 2.3% at 9.8 μg/l. There was no cross-reactivity with IGFBP-2, -3 and -4. Non-phosphorylated IGFBP-1 was measured by IRMA (DSL). This assay recognizes primarily non-phosphorylated IGFBP-1 isoforms, without reactivity to the highly phosphorylated variants. The sensitivity of the assay to the state of IGFBP-1 phosphorylation is such that, in serum samples from normal adults, the assay detects only 5–10% of the total IGFBP-1 measured by total IGFBP-1 IRMA (DSL) but registers a rapid rise in concentrations after sample treatment with alkaline phosphatase which dephosphorylates IGFBP-1. The sensitivity is 0.2 μg/l with inter- and intra-assay CV of 4.5 and 2.2% respectively at an analyte concentration of 12.0 μg/l (Khosravi et al., 1997).

Table I. Demographic characteristics of the women studied

<table>
<thead>
<tr>
<th></th>
<th>Controls (n = 10)</th>
<th>BN (n = 16)</th>
<th>IUGR (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean age (range)</td>
<td>31 (22–42)</td>
<td>27 (22–35) (NS)</td>
<td>34 (31–38) (NS)</td>
</tr>
<tr>
<td>Gestation at sampling</td>
<td>23+5 (22–25+5)</td>
<td>24+4 (23–25)</td>
<td>23+1 (22–26)</td>
</tr>
<tr>
<td></td>
<td>(NS)</td>
<td>(NS)</td>
<td>(NS)</td>
</tr>
</tbody>
</table>

BN = bilateral notches; IUGR = intrauterine growth restriction; NS = not significant at the 95% level.

For defining characteristics of BN and IUGR groups see text.
at 27 weeks of an infant weighing 500 g. There were three cases of pre-eclampsia in this group.

Discussion
There is evidence that excess IGFBP-1 in human maternal and fetal serum may contribute to growth restriction (Langford et al., 1993; Spencer et al., 1995) and transgenic animals overexpressing IGFBP-1 exhibit poor fertility and reproductive function (Gay et al., 1997).

In this study it was found that elevated maternal serum total IGFBP-1, within a narrow time frame between 22 and 26 weeks, was associated with IUGR seen in the second/third trimesters. Because the samples were taken within a small gestational range valid comparisons between the groups could be made. Hormone and peptide concentrations may vary according to gestational age, although from previous work it is known that maternal serum IGFBP-1 rises rapidly in the first trimester and then remains relatively stable throughout gestation (Westwood et al., 1994). This finding of elevated IGFBP-1 associated with IUGR has been reported by other workers but the different IGFBP-1 isoforms have often not been identified and the studies performed at different gestations throughout pregnancy and retrospectively (de Groot et al., 1996; Hills et al., 1996; Wang et al., 1996; Giudice et al., 1997). The data presented here showed that maternal serum IGFBP-1 was increased when the fetal Dopplers were abnormal and this may not be an early feature of the disease since cases that developed IUGR in the BN group appeared to have IGFBP-1 concentrations in the normal range.

The underlying pathology for impaired placentation is thought to be abnormal trophoblast invasion earlier in the pregnancy. Decidually derived IGFBP-1 is known to interact with trophoblast derived IGF-II in this regulation of invasiveness. Impaired placentation syndromes, such as IUGR, pre-eclampsia and abortion are characterized by abnormal trophoblast invasion and elevated IGFBP-1 might be expected in these scenarios. IGFBP-1 is generally inhibitory, causing both net suppression of trophoblast invasion and by binding with a higher affinity than IGF-1 and II for their receptors, providing a biological mechanism for maternal restraint on invasion by blastocyst derived trophoblast.

It is not known whether phosphorylation plays an in-vivo role in the regulation of IGFBP-1 action. However, since IGFBP-1 is known to be important in blastocyst implantation and trophoblast invasion, its isoforms and phosphorylation status are likely to be important. In this study uterine artery Doppler ultrasound was used in the late second trimester to identify women at higher risk of IUGR before clinical presentation in the early third trimester. It was postulated that differences in maternal serum concentrations of total IGFBP-1 and the ratio of the non-phosphorylated isoform to the total IGFBP-1 concentration might provide insight into the earlier pathology of impaired placentation and provide a marker for these important clinical syndromes.

The main site of synthesis of IGFBP-1 is the adult liver in the non-pregnant human, where it is phosphorylated before secretion. However, the predominant synthetic site in pregnant human is decidualized endometrium, where IGFBP-1 is thought to be dephosphorylated by alkaline phosphatases, before secretion as non- and partially phosphorylated forms (Westwood et al., 1994).

In this study it was found that both total and non-phosphorylated IGFBP-1 were significantly higher in the IUGR group compared with the control group. Non-phosphorylated IGFBP-1 is known to have decreased affinity for IGF; it would therefore be expected to have a less inhibitory action hence allowing increased IGF signalling at target receptors. In the IUGR group, raised absolute concentrations of non-phosphorylated IGFBP-1, with less binding affinity to IGF, would be advantageous since it would allow increased IGF bioavailability. Conversely there was a lower proportion of non-phosphorylated IGFBP-1 in the bilateral notch group compared to controls, a trend evident in the IUGR group. A lower proportion of non-phosphorylated IGFBP-1 in the total IGFBP-1 pool would imply an overall shift towards greater IGF binding affinity and decreasing IGF bioavailability. The combination of higher concentrations of total IGFBP-1, but lower ratios of non-phosphorylated:total IGFBP-1, in the high risk groups (BN and IUGR) was associated with conditions of impaired placentation, such as abortion, pre-eclampsia, fetal growth restriction and intrauterine death. It is not clear whether the rises in absolute concentrations of total and non-phosphorylated IGFBP-1 and the lower ratios of non-phosphorylated:total IGFBP-1 are primary or secondary compensatory phenomena.

The origin of the raised concentrations of total IGFBP-1 found in the IUGR group in this study may be due to decreased clearance by tissue uptake/serum degradation or, more likely, overproduction by dysfunctional decidua. The stimulus for increased IGFBP-1 production in impaired placentation syndromes such as IUGR and pre-eclampsia is currently not known. Whether it is up-regulation by decidual cells themselves or via stimulators and inhibitors of IGFBP-1 is unclear. Stimulators of IGFBP-1 synthesis include glucocorticoids, progesterone and activators of cyclic adenosine monophosphate. Inhibitors include interleukin-1ß, insulin and IGF. In the impaired placentation syndromes it is possible that the abnormal trophoblast-endometrial interface, with its increased vascular permeability and vasospasm, may account for the increased release of IGFBP-1 from the periarteriolar areas of the decidua into the maternal circulation. Elevated IGFBP-1 in cord serum in term babies with mixed respiratory/metabolic acidosis, indicating profound and prolonged hypoxia, has been reported. The authors suggest that hypoxia regulation of IGFBP-1 may restrict IGF mediated growth in utero under conditions of chronic hypoxia and limited substrate availability (Tazuke et al., 1998).

IGFBP-1 is one of a number of factors known to be important in placental physiology and intrauterine growth. From this study it is suggested that phosphorysoforms of IGFBP-1 in the maternal circulation might be associated with the pathological scenarios seen clinically, particularly in conditions thought to be due to impaired placentation. The possibility of identifying pregnancies at high risk of impaired placentation using Doppler ultrasound screening and strict clinical and ultrasound criteria early in the second trimester will allow...