The concentration of insulin-like growth factor-I and insulin-like growth factor-binding protein-1 in human umbilical cord serum at delivery: relation to fetal weight

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ABSTRACT

Serum levels of insulin-like growth factor-I (IGF-I) and insulin-like growth factor-binding protein-1 (IGFBP-1) have been determined by radioimmunoassay in the maternal circulation (n = 91) and in the umbilical artery (n = 56) and vein (n = 90) of man. In both the umbilical artery and vein, the concentration of serum IGF-I showed an inverse correlation with birthweight (P<0.005 and P<0.001 respectively); the mean serum IGF-I levels in the small-for-gestational-age (SGA) group were significantly higher than those in average-for-gestational-age (AGA) neonates (P<0.01 and P<0.001 respectively). However, maternal serum IGF-I showed no association with birthweight and there was no significant difference between the SGA and AGA groups. These observations imply that the production of IGF-I in the maternal and fetal compartments is independent and that there is unlikely to be transfer of IGF-I across the placenta. Serum IGFBP-1 levels in both maternal and umbilical cord blood (artery and vein) showed an inverse relation to birthweight (P<0.001, P<0.005 and P<0.001 respectively). Increased IGFBP-1 levels in the umbilical artery and vein were observed in the SGA group. These findings suggest that IGFBP-1 might inhibit the action of IGF-I in both the maternal and the fetal compartments and that the rise in IGFBP-1 could be a primary factor in retardation of fetal growth. Alternatively, circulating IGF-I and IGFBP-1 levels may only be a secondary reflection of local tissue events involved in fetal growth.


INTRODUCTION

Insulin-like growth factors I and II (IGF-I and IGF-II) are mitogenic peptides which are believed to be growth hormone (GH) dependent (Burin, Paterson, Sharp & Yeo, 1987). They can promote tissue growth and differentiation, and have insulin-like metabolic effects (Guler, Zapf & Froesch, 1987). These effects are mediated via two classes of receptors on target cell surfaces (Rechler & Nisley, 1985). Type I receptors also bind insulin at high concentrations (Ullrich, Gray, Tam et al. 1986); type II receptors bind only IGFs. The circulating levels of IGFs are higher than tissue concentrations; this is due to the presence of specific binding proteins (IGFBPs). The majority of IGFs in blood are bound to IGFBP-3, a large molecular mass (150 kDa) complex. A smaller proportion is bound to IGFBP-1. This has a molecular mass of 25 kDa and has proved to be identical with material isolated from the decidua (placental protein 12 and human pregnancy-associated endometrial α₂-globulin (Bohn & Kraus, 1980; Bell, Patel, Hales et al. 1985)). IGFBP-1 is not only a carrier protein in the circulation but also acts as a modulator of IGF action at the cellular level. Some investigators have shown that IGFBP-1 can inhibit the cellular response to IGFs (Ritvos, Ranta, Jalkanen et al. 1988) while others have suggested that it might enhance the action of IGFs (Elgin, Busby & Clemmons, 1987).

During pregnancy, maternal circulating IGF-I concentrations increase progressively (Wilson, Bennett, Adamson et al. 1982; Hall, Enberg, Hellen et al. 1984). In fetal blood, IGF-I can be detected from as early as 15 weeks of gestation (Ashton, Zapf, Einschenk & MacKenzie, 1985). At term, fetal IGF-I levels show a positive correlation with birthweight and gestational age (Wilson et al. 1982; Gluckman, Johnson-Barrett, Butler et al. 1983; Ashton et al. 1985). In the fetal rat, there is a decrease in IGFs following experimental growth retardation produced by ligation of uterine
vessels (De Prins, Hill, Fekete et al. 1984). Similar observations have been made in man; cord serum IGF levels from infants with intrauterine growth retardation are reduced (Foley, De Philip, Perricelli & Miller, 1980). However, IGFBP-1 levels in the maternal circulation reveal an inverse correlation with delivered weight, being increased in pregnant subjects who deliver low-birthweight infants (Howell, Perry, Choglay et al. 1985; Hall, Hasson, Lundin et al. 1986). Furthermore, the serum concentrations of IGFBP-1 are also increased in pregnancies complicated by proteinuric pre-eclampsia, a condition that is frequently associated with intrauterine growth retardation (Howell, Economides, Teisner et al. 1989).

We now report the concentrations of IGF-I and IGFBP-1 in the maternal circulation and in the umbilical artery and vein at delivery. The relationship between IGF-I and IGFBP-1 levels and birthweight has been further explored.

MATERIALS AND METHODS

Subjects

Maternal blood samples (10 ml) were collected from an antecubital vein at the time of delivery in 91 pregnant women (84 with neonates whose birthweight was average-for-gestational age (AGA); and seven considered small-for-gestational age (SGA, birthweight below the tenth percentile for gestational age; Golde, 1989)). Maternal ages ranged from 20 to 34 years. The study had been approved by the local Ethical Committee and informed consent was obtained from each woman. All subjects had a full-term uneventful pregnancy (38–42 weeks) and normal vaginal delivery. Blood samples (5–10 ml) were also collected from the umbilical artery (n = 56) and vein (n = 90). All the samples were collected from 07.00 h to 23.00 h, avoiding the nocturnal peak of circulating IGFBP-1 levels which rise 10–20-fold between 24.00 h and 06.00 h (Baxter & Cowell, 1987). Serum was stored at −20°C until assay.

Radioimmunoassay (RIA) of IGFBP-1

Serum levels of IGFBP-1 were measured by an RIA as previously described (Wang, Perry, Kanisius et al. 1991). Tracer was prepared by iodination of IGFBP-1 using chloramine T. A polyclonal antiserum to IGFBP-1 (lot no. S515 Wang et al. 1991) was used at a final dilution of 1:500 000 which bound approximately 50% of [125I]-labelled IGFBP-1. The sensitivity of the assay, defined as the smallest concentration that could be distinguished from the zero standard, was 5 μg/l. The intra-assay coefficients of variation were 4.5% at 50 μg/l and 3.7% at 105 μg/l. The interassay coefficients of variation were 8.8% at 50 μg/l and 7.4% at 105 μg/l.

Production of antisem to IGF-I

Recombinant human IGF-I (lot no. CGP 35126), kindly provided by Drs W. Marki and K. Scheibeli (Ciba-Geigy Limited, Basel, Switzerland), was used for immunization of three sheep. Protein (250 μg) dissolved in 1 ml saline and emulsified in 2.5 ml complete Freund's adjuvant was injected s.c. into the neck at six sites for the first immunization. Animals were given booster injections at intervals of 4 weeks with 150 μg IGF-I emulsified in incomplete Freund's adjuvant. The first bleed was taken 2 weeks after the first booster injection; thereafter the sheep were sampled every 4 weeks.

RIA of IGF-I

Recombinant human IGF-I (5 μg) was reacted with 1 mCi Na125I (Amersham International plc, Amersham, Bucks, U.K.) and 5 μg chloramine T in a final volume of 50 μl phosphate buffer (0.05 mol/l; pH 7.4). After 15 s of mixing, the reaction was terminated by addition of 5 μg sodium metabisulphite followed by 300 μl phosphate buffer (0.05 mol/l; pH 7.4) containing 1% (w/v) bovine serum albumin (BSA). Separation of non-incorporated iodide from labelled IGF-I was achieved by chromatography on a 0.9 cm × 30 cm column of Sephadex G-25 (Superfine; Pharmacia, Uppsala, Sweden) eluted with phosphate buffer (0.05 mol/l; pH 7.4) containing 1% BSA.

Prior to RIA for IGF-I, serum samples were treated by acid-ethanol extraction to dissociate IGF-I from binding proteins (Daughaday, Ward, Goldberg et al. 1982). Briefly, 0.2 ml serum was extracted with 0.8 ml 87.5% (v/v) ethanol and 12.5% (v/v) 2 mol HCl/l at room temperature for 30 min. After centrifugation at 1850 g for 30 min at 4°C, 0.5 ml supernatant was transferred and neutralized with 0.2 ml 0.86 mol Tris base/l.

The IGF-I RIA was carried out in phosphate buffer (0.05 mol/l; pH 7.4) containing 5% BSA. Initially, 100 μl standard or acid–ethanol-extracted sample were preincubated with 100 μl antiserum (lot no. S560; final dilution 1:240) and 250 μl running buffer. After 2 h, 50 μl [125I]-labelled IGF-I (3000 cpm; containing approximately 8 pg [125I]-labelled IGF-I; prepared as described above) was added and the incubation continued for another 20 h at 4°C. Separation of antibody–ligand complex from free IGF-I was performed by incubation of 50 μl donkey anti-sheep antiserum (final dilution 1:240), 50 μl normal sheep serum (final dilution 1:4800) and 1 ml 20% (w/v) polyethylene glycol (M, 8000; Sigma, St Louis, MO, U.S.A.) for 30 min at room temperature followed by centrifugation at 2000 g for another 30 min. Following
removal of supernatants, the radioactivity in the pellets was counted in a gamma counter (Hydragramma 16, Innatron Ltd, Oxford, Oxon, U.K.). Unlabelled recombinant human IGF-I (Drs W. Marki and K. Scheibli; Ciba-Geigy Ltd) at concentrations between 5 and 1000 µg/l was used to obtain a standard curve. The minimum detection limit of the assay was 10 µg/l. Nonspecific binding (NSB) of 125I-labelled IGF-I in the absence of antiserum was 3-5-5-5%. The intra-assay coefficients of variation were 4-9% at 345 µg/l and 10-2% at 185 µg/l. The interassay coefficients of variation (n = 8) were 7-1% at 345 µg/l and 13-4% at 185 µg/l. There was no cross-reaction with insulin (Sigma), IGF-II (Ciba-Geigy Ltd) or IGFBP-1, purified as previously described (Wang et al. 1991).

RESULTS

In umbilical cord blood, both arterial and venous IGF-I levels showed an inverse correlation with birthweight (P < 0.005 and P < 0.001 respectively) (Fig. 1). The mean concentration of umbilical cord serum IGF-I in the SGA group was significantly higher than that in AGA infants (Table 1). By contrast, no association was found between maternal serum IGF-I levels

<table>
<thead>
<tr>
<th>Analyte</th>
<th>SGA (µg/l) (range; n)</th>
<th>AGA (µg/l) (range; n)</th>
<th>SGA vs AGA (Fisher’s exact probability test)</th>
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</thead>
<tbody>
<tr>
<td>MS IGF-I</td>
<td>196 (105–322; 7)</td>
<td>196 (35–1092; 84)</td>
<td>NS</td>
</tr>
<tr>
<td>UV IGF-I</td>
<td>756 (105–1050; 7)</td>
<td>154 (35–994; 83)</td>
<td>P = 0.005*</td>
</tr>
<tr>
<td>UA IGF-I</td>
<td>658 (119–945; 5)</td>
<td>182 (35–190; 51)</td>
<td>P = 0.0165*</td>
</tr>
<tr>
<td>MS IGFBP-1</td>
<td>115 (73–1200; 7)</td>
<td>175 (23–1100; 84)</td>
<td>NS</td>
</tr>
<tr>
<td>UV IGFBP-1</td>
<td>350 (59–1200; 7)</td>
<td>122 (18–900; 83)</td>
<td>P = 0.0198*</td>
</tr>
<tr>
<td>UA IGFBP-1</td>
<td>380 (81–1100; 5)</td>
<td>142 (20–660; 53)</td>
<td>P = 0.0214*</td>
</tr>
</tbody>
</table>

*The 90th percentile of IGF-I levels in the AGA group was used as the cut-off value in Fisher’s exact probability test.
†The 75th percentile of IGFBP-1 levels in the AGA group was used as the cut-off value in Fisher’s exact probability test.
NS, non-significant.

FIGURE 1. The relationship between (a) IGF-I levels in the umbilical artery (UA) and birthweight (BW) (n = 54), (b) IGF-I levels in the umbilical vein (UV) and BW (n = 90), (c) IGF-binding protein-1 (IGFBP-1) levels in the maternal serum (MS) and BW (n = 91), (d) IGFBP-1 levels in the UA and BW (n = 56) and (e) IGFBP-1 levels in the UV and BW (n = 90).

TABLE 1. The concentration of IGF-I and IGFBP-1 (IGFBP-1) (median and range) in maternal venous serum (MS), umbilical vein (UV) and umbilical artery (UA). Serum levels in the small-for-gestational-age (SGA) and average-for-gestational-age (AGA) group are shown.
and birthweight and maternal circulating IGF-I levels in the SGA group were similar to those in the AGA group (Table 1). In the AGA group there was no difference in IGF-I levels between maternal serum and cord arterial and venous serum; in the SGA group, mean IGF-I levels in the umbilical artery and vein were substantially higher than those in maternal serum, but this difference was not significant \((P>0.05\), Fisher’s exact probability test).

In both maternal and umbilical cord blood (artery and vein), IGFBP-1 levels showed a significant inverse correlation with birthweight \((P<0.001, P<0.005\) and \(P<0.001\) respectively) (Fig. 1). Cord serum IGFBP-1 was higher in SGA neonates than in the AGA group, but maternal IGFBP-1 showed no significant difference between the two birthweight groups (Table 1). In the SGA group, the levels of IGFBP-1 in the cord artery and vein were substantially higher than those in the maternal circulation, but this difference was not significant \((P>0.05\), Fisher’s exact probability test).

In all situations the concentration of IGF-I and IGFBP-1 in the umbilical artery was similar to that in the umbilical vein and there was a close association between them \((P<0.0001)\) (Table 2). In both maternal and umbilical cord serum (artery and vein), IGFBP-1 levels were positively correlated with IGF-I levels \((P<0.01, P<0.0005\) and \(P<0.0001\), respectively). Maternal serum IGFBP-1 concentrations were related to those in the umbilical artery and vein \((P<0.005\) and \(P<0.02\) respectively). There was no correlation between maternal serum IGF-I and umbilical arterial IGF-I levels; maternal IGF-I levels were positively associated with those in umbilical vein \((P<0.05)\).

**DISCUSSION**

The present results confirm previous findings that maternal serum IGFBP-1 levels are inversely associated with birthweight (Howell et al. 1985; Hall et al. 1986). They also demonstrate, for the first time, that the same association is seen in fetal serum. Thus, if the functional significance of the increased IGFBP-1 is to inhibit the action of IGF-I, then this phenomenon applies equally in both the maternal and the fetal compartments.

The bulk of the circulating IGFBP-1 in pregnancy is believed to be synthesized by decidualized endometrium (Rutanen, Menabawey, Isaka et al. 1986; Bell, 1989). It has been proposed that the increased IGFBP-1 may protect the endometrium from further invasion by trophoblast, acting via inhibition of IGF-I receptors in placental membranes and thereby restricting IGF-I action (Pekonen, Suikkari, Makinen & Rutanen, 1988). It would be attractive to speculate that the maternal factor in the control of fetal growth is partly mediated by the decidua, acting via synthesis of IGFBP-1. However, the present studies show that the relationship between delivered weight and both IGFBP-1 and IGF-I is more striking in the fetal circulation than in the maternal circulation (Table 1). Since the only route by which decidual products could reach the fetus is via the maternal circulation and placental transfer, it seems unlikely that the decidua can play a primary role.

In the present study, IGF-I levels in both umbilical artery and vein were inversely related to birthweight, and cord serum IGF-I levels were significantly increased in the SGA group. These findings are in marked contrast to those of previous investigators, most of whom have reported that IGF-I levels are relatively low in the fetal circulation and show a positive correlation with birthweight (Wilson et al. 1982; Gluckman et al. 1983; Ashton et al. 1985). Reduced IGF levels have also been shown in cord serum from human SGA neonates (Foley et al. 1980; Aleem, Moharam, Schulman et al. 1990) and in fetal rats with experimentally induced growth retardation (De Prins et al. 1984). The reason for this discrepancy is not obvious. However, the concomitant high levels of serum IGFBP-1 in SGA neonates may inhibit the effect of IGF-I on tissue growth and result in growth

**TABLE 2. Correlation of IGF-I and IGFBP-1 (IGFBP-1) levels between maternal serum (MS) and umbilical cord (UV, umbilical vein; UA, umbilical artery)**

<table>
<thead>
<tr>
<th></th>
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<th>Correlation</th>
<th>Equation ((y=))</th>
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<tbody>
<tr>
<td>UV IGF-I</td>
<td>UA IGF-I</td>
<td>Positive</td>
<td>38.52 + 0.8939x</td>
<td>0.8723</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>UV IGFBP-1</td>
<td>UA IGF-I</td>
<td>Positive</td>
<td>26.93 + 0.9892x</td>
<td>0.90</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>UV IGF-B</td>
<td>UV IGF-I</td>
<td>Positive</td>
<td>75.46 + 0.7946x</td>
<td>0.7233</td>
<td>&lt;0.0001</td>
</tr>
<tr>
<td>UA IGF-B</td>
<td>UA IGF-I</td>
<td>Positive</td>
<td>79.60 + 0.7928x</td>
<td>0.6421</td>
<td>&lt;0.0005</td>
</tr>
<tr>
<td>MS IGF-B</td>
<td>MS IGF-I</td>
<td>Positive</td>
<td>169.15 + 0.2152x</td>
<td>0.2745</td>
<td>&lt;0.01</td>
</tr>
<tr>
<td>MS IGF-B</td>
<td>MS IGF-I</td>
<td>Positive</td>
<td>161.72 + 0.2839x</td>
<td>0.23</td>
<td>&lt;0.05</td>
</tr>
<tr>
<td>MS IGF-B</td>
<td>UA IGF-I</td>
<td>NS</td>
<td>134.42 + 0.23x</td>
<td>0.254</td>
<td>&lt;0.02</td>
</tr>
<tr>
<td>MS IGF-B</td>
<td>MS IGF-I</td>
<td>Positive</td>
<td>123.82 + 0.32x</td>
<td>0.41</td>
<td>&lt;0.005</td>
</tr>
</tbody>
</table>

NS, non-significant.
retardation. Alternatively, the high levels of circulating IGF-I in SGA infants might be the result of an increase in serum IGFBP-1, thereby protracting the half-life and delaying the clearance of circulating IGF-I.

It is essential to dissociate serum IGF-I from its binding proteins by acid–ethanol extraction prior to RIA. Thus, serum IGF-I levels may not reflect the entire situation of fetal growth. A bioassay might be more appropriate for assessment of the effect of circulating IGF-I on the control of fetal growth. Because of this, RIA measures total IGF-I, not the free unbound fraction which may be responsible for biological activity.

The fact that IGF-I levels were similar in arterial and venous cord serum suggests that IGF-I is synthesized within the human fetus itself. This concept is supported by the demonstration of IGF messenger RNAs in a wide range of human fetal tissues (Han, D'Ercole & Lund, 1987), the observation that many fetal tissues can secrete IGF-I in organ culture (Hill, Crace, Nissley et al. 1985; Swenne, Hill, Strain & Milner 1987) and the wide distribution of IGFBPs in fetal tissues (Hill, Clemmons, Wilson et al. 1989). The latter studies further imply that IGF-I may stimulate growth via autocrine or paracrine actions at the site of production.

During pregnancy there is a progressive increase in circulating levels of IGF-I; this appears to be independent of pituitary GH secretion (Wilson et al. 1982; Hall et al. 1984). It is possible that the increased IGF-I plays a role in the regulation of placental growth; this is supported by the observation that cultured placental stromal cells respond to exogenous IGF-I (Fant, Munro & Moses, 1986). The rapid decline in IGF-I levels postpartum (Franklin, Pepperell, Renne & Cameron, 1979) suggests that the placenta and/or the fetus may secrete substances which stimulate maternal IGF-I production. Candidates include placental lactogen and, in particular, placental GH (Frankenne, Closset, Gomez et al. 1988). However, the present results show only a weak association between maternal and umbilical cord serum IGF-I levels, and no relationship between maternal serum IGF-I levels and fetal birthweight. This implies that the production of IGF-I in the maternal and fetal compartments is independent and that there is unlikely to be significant transfer of IGF-I across the placenta.

The fact that IGFBP-1 and IGF-I are identically distributed in human fetal tissues during the second trimester suggests that the action of IGF-I on tissue growth and differentiation may be modulated by IGFBP-1 at the tissue level (Hill et al. 1989). This is further supported by the observation that, prior to 26 weeks gestation, the major binding protein for IGF-I in fetal serum is IGFBP-1 (D'Ercole, Wilson & Underwood, 1980). However, although the main fetal IGFBP after 30 weeks gestation is the GH-dependent IGFBP-3 (D'Ercole et al. 1980), the serum concentrations of IGFBP-1 are now shown to be higher in SGA neonates. This and the inverse relation to birthweight are consistent with an important inhibitory role of IGFBP-1 on IGF action and growth of the fetus in utero. Alternatively, both IGFs and their binding proteins may be intermediate paracrine or autocrine factors in the process of growth control, under the control of a more primary but as yet unidentified endocrine factor (Chard, 1989).

If the present demonstration of increased levels of IGF-I in association with SGA is correct, then it is possible that this is secondary to the increase in IGFBP-1. The mechanism would be the same as that which applies to some steroids and steroid-binding proteins in pregnancy (e.g. cortisol-binding globulin leads to an increase in the total circulating cortisol). The overall effect is association of low-birthweight infants with high circulating IGF-I and IGFBP-1 levels. If circulating bound IGF-I is biologically inactive and cannot bind to receptors on fetal tissues, the rise of IGFBP-1 could be the primary cause of growth retardation.

Whether circulating IGF-I and IGFBP-1 play a direct role in fetal growth still requires further investigation. Thus, while it is very likely that fetal growth is influenced by an interaction amongst IGF-I, IGFBP-1 and IGF-I receptors at the local tissue level, the circulating concentrations of IGF-I and IGFBP-1 may only indirectly reflect these events.

REFERENCES


