Decreased maternal circulating hepatocyte growth factor (HGF) concentrations in pregnancies with small for gestational age infants

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Our purpose was to evaluate whether maternal and fetal hepatocyte growth factor (HGF) concentrations in pregnancies with small for gestational age (SGA) infants are different from those in pregnancies with appropriate for gestational age (AGA) infants. Maternal and fetal circulating HGF concentrations were measured between 55 pregnancies with AGA infants and 16 pregnancies with SGA infants at birth. HGF concentrations were measured from maternal and cord venous blood samples using an enzyme-linked immunosorbent assay. Umbilical artery blood pH and oxidative pressure (PO2) were also measured. Maternal circulating HGF concentrations (0.60 ± 0.35 ng/ml) in pregnancies with SGA infants were significantly lower than those (0.91 ± 0.44 ng/ml) in pregnancies with AGA infants (P = 0.012). There were no significant differences in fetal circulating HGF concentrations between both groups. No significant differences in umbilical artery blood pH and PO2 were found between both groups. These results suggest that the maternal serum circulating HGF concentration has a significant role in fetal growth during pregnancy.

Key words: appropriate for gestational age infant/fetal growth/hepatocyte growth factor/small for gestational age infant

Introduction

Fetal growth restriction remains one of the major problems currently facing obstetricians because it contributes to perinatal morbidity and mortality. Other than fetal insulin, none of the major endocrine hormones seems to have a direct influence on fetal growth. Recent biomolecular data suggested that locally synthesized macromolecules, acting either in an autocrine or paracrine manner, regulate embryonic organ and tissue growth (Selden et al., 1990).

Hepatocyte growth factor (HGF) was originally identified as a factor that stimulates mitogenesis of hepatocytes (Nakamura et al., 1984), and noted as a potent mitogen for normal human keratinocyte, melanocyte, and renal tubular epithelial cells (Igawa et al., 1991; Matsumoto et al., 1991a,b). In non-pregnant women, HGF was produced in mesenchyme-derived cells such as fibroblasts, Kupffer cells, macrophages and endothelial cells (Kinoshita et al., 1989; Noji et al., 1990). Recently, it has been reported that both placenta and amniotic membrane produce and secrete HGF (Wolf et al., 1991; Clark et al., 1996), and in pregnant woman, maternal HGF is mainly released from the placenta into the maternal circulation (Horibe et al., 1995).

In the fetus, a major site for producing HGF is thought to be the liver, as well as the kidney and pancreas (Selden et al., 1990). It has been verified that HGF has an important physiological role for fetal growth including liver regeneration and development, and differentiation of the placenta (Selden et al., 1990; Uehara et al., 1995). However, little information currently exists regarding the role of HGF in appropriate for gestational age (AGA) and small for gestational age (SGA) infants during pregnancy. The aim of this study was to compare maternal and fetal sera HGF concentrations between pregnancies with AGA infants and those with SGA infants, to determine whether pregnancies with SGA infants demonstrate lower maternal and fetal sera circulating HGF concentrations.

Materials and methods

Maternal and fetal circulating HGF concentrations were compared between 55 pregnancies with AGA infants and 16 pregnancies with SGA infants at birth. These women were non-smokers, with neither indication of maternal complication nor incidence of drug administration. Those subjects with liver impairment, diabetes, multiple pregnancies, fetal hydrops, pre-eclampsia, previous pregnancy with pre-eclampsia or molar pregnancies were excluded from the study. Clinical characteristics of the subjects are given in Table I. Gestational age was estimated from the first day of the last menstrual period and was confirmed by first-trimester and early second trimester ultrasound examinations (crown–rump length, biparietal diameter and

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| Table I. Clinical characteristics of subjects of the appropriate for gestational age (AGA) and small for gestational age (SGA) groups of infants. Data are shown as mean ± SD |

<table>
<thead>
<tr>
<th>Maternal age (years)</th>
<th>AGA (n = 55)</th>
<th>SGA (n = 16)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maternal age (years)</td>
<td>28.8 ± 4.1</td>
<td>28.9 ± 4.0</td>
</tr>
<tr>
<td>Parity</td>
<td>0.9 ± 0.8</td>
<td>1.0 ± 0.9</td>
</tr>
<tr>
<td>Birth age (weeks)</td>
<td>39.5 ± 1.1</td>
<td>39.1 ± 1.4</td>
</tr>
<tr>
<td>Birth weight (g)</td>
<td>3128 ± 257</td>
<td>2450 ± 358</td>
</tr>
<tr>
<td>Apgar score at 1 min</td>
<td>8.7 ± 0.8</td>
<td>7.7 ± 1.9</td>
</tr>
<tr>
<td>Umbilical artery blood gas analysis</td>
<td></td>
<td></td>
</tr>
<tr>
<td>pH</td>
<td>7.27 ± 0.06</td>
<td>7.27 ± 0.04</td>
</tr>
<tr>
<td>PO2 (mm Hg)</td>
<td>17.1 ± 6.1</td>
<td>14.8 ± 6.7</td>
</tr>
</tbody>
</table>

PO2 = oxidative pressure.

Values with same superscript are significantly different from each other (P < 0.05).
femur length measurements). The patients were allocated into AGA and SGA groups after birth weight measurement.

Estimated fetal weights by ultrasound during pregnancy and birth weights in AGA group were within normal ranges (10–90th percentile) of the standard growth curve for the Japanese (Sato et al., 1982), and those in SGA group below normal ranges (<10th percentile). The umbilical artery pulsatility index in the AGA group was within normal ranges (Manabe et al., 1995). In two out of 16 patients in the SGA group, the umbilical artery pulsatility index was >95th percentile, and that of the other 14 was within the normal range. The gestational age at delivery of all subjects in this study ranged between 37 and 41 weeks of pregnancy. All AGA group babies were delivered vaginally. Two babies were delivered by Caesarean section due to fetal distress, and 14 by vaginal delivery in pregnancies with SGA infants. In all 16 pregnancies with SGA infants, there was no known cause for the SGA. No neoate showed a congenital malformation or other genetic disorder. The study was approved by the local ethical committee of Shimane Medical University, Japan, and standardized informed consent was obtained from each patient.

After delivery of the placenta, 10 ml samples of the maternal blood and cord blood were obtained. These were performed by needle puncture of the maternal cubital vein and umbilical vein. Samples of the maternal blood were collected at delivery from 50 patients in the AGA group and 15 in the SGA group. Specimens were then centrifuged (1000 g for 10 min), and serum was separated and stored at –80°C until assayed. Simultaneously, a 1 ml sample of umbilical artery blood was obtained, and blood gas analysis was then performed on a Ciba Corning 278 (Ciba Corning Diagnostic Co Ltd, Tokyo, Japan) pH blood gas analyser.

HGF was measured by an enzyme-linked immunosorbent assay (ELISA) kit (HGF Otsuka ELISA kit; Otsuka Pharmaceutical Co. Ltd, Tokyo, Japan). The minimum detection limit of this assay for HGF was 0.3 ng/ml. The coefficients of intra-assay and inter-assay variation were 3.7 and 4.7% respectively.

Statistical analysis
The unpaired t-test was used to compare birth age, birth weight, umbilical artery blood pH and oxidative pressure (PO$_2$) and maternal and fetal circulating HGF concentrations between the AGA and SGA groups. For comparison of maternal age, parity, and Apgar score between the two groups, the Wilcoxon–Mann–Whitney test was used and the Spearman rank test was used to assess correlation between maternal and fetal HGF concentrations from the same patients. $P < 0.05$ was considered to be significant. All data are expressed as mean ± SD.

Results
There were no significant differences for maternal age, parity, birth age, and umbilical artery blood pH and PO$_2$ between AGA and SGA groups. There was no significant difference in maternal body mass index (BMI) in the early stages of pregnancy or at delivery between AGA and SGA groups (early stages of pregnancy; 22.4 ± 3.2 versus 23.6 ± 2.9, $P = 0.35$; compared with 24.8 ± 3.0 versus 26.1 ± 2.7, $P = 0.28$ at delivery). Birth weight (2450 ± 358 g) and Apgar score at 1 min (7.7 ± 1.9) in SGA group were significantly lower than those (3128 ± 257 g and 8.7 ± 0.8) in AGA group respectively ($P < 0.05$) (Table I).

Maternal circulating HGF concentrations (0.60 ± 0.35 ng/ml) in the SGA group were significantly lower than those (0.91 ± 0.44 ng/ml) in the AGA group ($P = 0.012$) (Figure 1). There were no significant differences in fetal circulating HGF concentration between the AGA (0.34 ± 0.30 ng/ml) and the SGA (0.33 ± 0.34 ng/ml) groups. There was a significant difference in the placental weight between AGA and SAG groups (628 ± 94 g versus 475 ± 75 g, $P < 0.001$).

There was a linear correlation between maternal and fetal circulating HGF concentrations from the same patients in the AGA group ($R^2 = 24.1\%$, $P = 0.037$). However, no significant correlation was observed between maternal and fetal circulating HGF concentrations from the same patients in the SGA group.

Discussion
HGF has a potential to stimulate directed and random migration of endothelial cells (Mimomoto et al., 1991), angiogenesis in the cornea (Bussolin et al., 1992), growth of gastric and intestinal cells (Fukamachi et al., 1994), and the growth and differentiation of multipotent and erythroid haematopoietic progenitor cells (Mizuno et al., 1993). Recently, it has been reported that in ‘knockout’ mice in which the HGF gene has been inactivated by targeted disruption the embryo fails to develop and dies in utero due to a marked loss of liver parenchymal cells and impairment of trophoblast cell development in the placenta (Schmidt et al., 1995; Uehara et al., 1995). Therefore, HGF would appear to play an essential role for normal liver and placental development in utero which is reflected in fetal systemic growth.

The human placenta is one of the richest sources of HGF (Wolf et al., 1991), which was first sequenced from a human placental cDNA library (Miyazawa et al., 1994). Schmidt et al. (1995) and Uehara et al. (1995) demonstrated independently that HGF is essential for normal placenta in mice. Absence of either HGF or c-met seems to prevent the normal growth and development of the epithelioid-derived labyrinthine trophoblast cells, leading to growth restriction and intrauterine death secondary to placental insufficiency (Somerset et al., 1997).
In our study, maternal circulating HGF concentrations in pregnancies with SGA infants were significantly lower than those in pregnancies with AGA infants. One possible explanation for this difference is that lower concentrations of maternal HGF in SGA pregnancy are secondary to the decrease of placental release of HGF into the maternal circulation. Most intrauterine growth restrictions are accompanied by characteristic pathological placental changes which cause inadequate substrate and oxygen supply to the fetus. In such pathological placentae, the expression of HGF is reduced as shown by immunohistochemistry (Li et al., 1996). Another explanation is that lower HGF concentrations in the SGA group might be due to decreased production of HGF from the small placenta.

The maternal liver is another possible site for HGF production. In this study, there is no significant difference in both biochemical liver function data and BMI between mothers in the AGA and SGA groups. Therefore, production of HGF in the maternal liver could not be considered.

HGF concentrations in maternal serum are attributed to HGF released from the placenta into the maternal circulation (Horibe et al., 1995). Therefore, in pregnancies with SGA, the release of HGF from the placenta into the maternal circulation is restricted and maternal concentrations are lower than those in pregnancies with AGA. On the other hand, maternal nutritional factors influence the birthweight of infants. In our study, there was no significant difference in maternal BMI in either the early stages of pregnancy or delivery between the AGA and SGA groups. Therefore, the growth restriction of the SGA fetus in our study was not secondary to maternal nutritional factors. Teenage pregnancy is also a cause of SGA infants (Fraser et al., 1995; Lao and Ho, 1997). In our study, there was no significant difference in the maternal ages between AGA and SGA groups. Moreover, no teenage pregnancy was included in this study.

With respect to fetal circulating HGF, the major productive organs were considered to be liver, kidney, pancreas, and lung (Selden et al., 1990). In addition, there is little, if any, release of HGF from the placenta into the fetal circulation (Horibe et al., 1995). In this study, umbilical cord HGF concentrations did not differ between AGA and SGA infants. Kahn et al. (1996) also reported that cord HGF does not differ at term between AGA and SGA infants. These results may suggest that fetal circulating HGF plays a less important role in fetal growth at term. The reason why there is no significant difference of fetal HGF concentrations between the AGA and SGA groups, despite the significant difference of maternal HGF concentrations between the two groups, is currently unknown. The major productive organ of fetal HGF is the liver. Senoh et al. (1994) found that the fetal liver length is normal in 90.5% of SGA fetuses. Therefore, one possible explanation is that the liver of SGA fetuses might produce a similar amount of HGF as the AGA fetus. On the other hand, Khan et al. (1996) found that, in premature deliveries, higher concentrations of cord HGF are noted in complicated pregnancies. This implies the possibility of a significant increase of fetal HGF under the condition of severe intrauterine stress. In our study, the stress in SGA infants seems to be similar with that in AGA infants, because there were no significant differences in umbilical artery blood pH and PO₂ between SGA and AGA infants. Fetal HGF concentrations under severe intrauterine stress may significantly change even at term. However, further study is needed to clarify the role of fetal circulating HGF concentrations in high risk pregnancies.

With respect to the relationship between maternal and fetal circulating HGF concentrations, a positive linear correlation was noted in pregnancies with AGA infants, whereas no correlation was noted in pregnancies with SGA infants. The reason for the difference in the relationship of maternal and fetal circulating HGF concentrations between AGA and SGA infants is currently unknown. Khan et al. (1996) suggested that cord HGF concentrations are high and relate to gestation in normal pregnancies, whereas there is no relationship between gestation and cord HGF in complicated pregnancies, including growth restricted infants. One possible explanation is that compensatory HGF secretion may occur in SGA fetuses. However, in view of the small number of SGA infants, these observations must be considered preliminary.

In summary, the current study demonstrated that maternal serum HGF concentrations were decreased in pregnancies with intrauterine growth restricted infants. The decreased HGF concentrations in maternal circulation in pregnancies with intrauterine growth restricted infants may reflect decreased HGF release from the placenta.

References


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