Hepatocyte growth factor concentration in the early second-trimester amniotic fluid does not predict fetal growth at birth

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The purpose of this study was to evaluate whether hepatocyte growth factor (HGF) concentrations in the early second-trimester amniotic fluid predict fetal growth at birth. HGF and insulin-like growth factor-I (IGF-I) concentrations in the early second-trimester amniotic fluid were measured in 12 pregnancies with small for gestational age (SGA) infants, 84 pregnancies with appropriate for gestational age (AGA) infants, and eight pregnancies with large for gestational age (LGA) infants. HGF concentrations were measured from the early second-trimester amniotic fluid samples using an enzyme-linked immunosorbent assay. IGF-I concentrations were measured from the early second-trimester amniotic fluid samples using an immunoradiometric assay. Maternal age in AGA group (34.2 ± 5.5 years) was significantly lower than in SGA (37.9 ± 3.0 years) and LGA (37.6 ± 3.3 years) groups (P < 0.05). There were no significant differences for parity or gestational age at amniocentesis among the groups. There were significant differences for birth age, birth weight, neonatal height, and placental weight among the groups (P < 0.05). HGF concentrations in SGA, AGA and LGA groups were 16.9 ± 6.6, 16.7 ± 9.0 and 20.2 ± 14.8 ng/ml respectively (not significant). There was no correlation between amniotic fluid HGF concentrations and birth weight, height or placental weight. There were also no significant differences for amniotic fluid IGF-I concentrations among the three groups. These results suggest that differences in HGF concentrations in the early second-trimester amniotic fluid do not predict fetal growth at birth. Further study is needed to clarify the role of high HGF concentrations in early second-trimester amniotic fluid during pregnancy.

Key words: amniotic fluid/early second-trimester/fetal growth/ hepatocyte growth factor/insulin-like growth factor-I

Introduction

Hepatocyte growth factor (HGF) is a ubiquitous and pluripotent cytokine with a variety of paracrine and endocrine effects (Strain et al., 1982), and 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). Specific expression of HGF is increasingly implicated in the regulation of placental growth and function, and thereby fetal growth and development. The spatial distribution of HGF and c-met within the human placenta suggests potential physiological actions, including angiogenesis and trophoblast growth (Somerset et al., 1997). As such functions are important in placental development and consequently fetal well-being, it remains to be determined whether inappropriate expression or over-expression of this glycoprotein or its receptor might lead to pregnancy complications such as fetal growth restriction or macrosomia.

Concentrations of HGF in the amniotic fluid were highest between 20 and 29 weeks of gestation, and amniot in the second-trimester was shown to secrete 100-fold more HGF than that from the third-trimester (Horibe et al., 1995). There has been only one report on the concentration of HGF in human amniotic fluid at second-trimester, in relation to fetal birth weight (Kurauchi et al., 1995). In that study, the concentration of amniotic HGF at second-trimester showed a significant inverse correlation with birth weight (r² = 20%, P < 0.05). However, the number of observations (n = 20) reported in that study was very small, and the value of coefficient of determination was low. The aim of this study was to re-evaluate whether amniotic fluid HGF concentrations at second-trimester show a significant inverse correlation with birth weight, and to evaluate whether HGF concentrations in the early second-trimester amniotic fluid predict fetal growth at birth.

Materials and methods

The early second-trimester amniotic fluid HGF and IGF-I concentrations (consecutive samples were obtained from amniocentesis for fetal chromosome analysis done between 14 and 20 weeks of gestation) were compared among 12 pregnancies with small for gestational age (SGA) infants, 84 pregnancies with appropriate for gestational age (AGA) infants, and eight pregnancies with large for gestational age (LGA) infants. These women were non-smokers, with neither indication of maternal complication nor incidence of drug administration. Those subjects with diabetes, multiple pregnancies, fetal hydrops, pre-eclampsia, previous pregnancy with pre-eclampsia or mole pregnancies were excluded from the study. Clinical characteristics for the subjects are given in Table I. Gestational age was estimated from the first day of the last menstrual period and confirmed by first-trimester and early second-trimester ultrasound examinations (crown–rump length, biparietal diameter and femur
length measurements). Birth weights in SGA infants were below normal ranges (below the 10th percentile), those in AGA infants within normal ranges (between the 10th and 90th percentile), and those in LGA infants above normal ranges (above the 90th percentile) to the standard growth curve for the Japanese (Sato et al., 1982). Most women were delivered at other hospitals, but in these instances the doctors in charge reported information on the child’s date of birth, birth age, sex, birth weight, height, placental weight, and maternal complications during pregnancy. Only one fetus in the SGA group was delivered by Caesarean section due to fetal distress, and all other fetuses were delivered vaginally. Therefore, in the absence of a true measure of growth rate, it was assumed that babies in the SGA group were normal small babies and not suffering from growth restriction. In no neonate was there congenital malformation or genetic disorder. The study was approved by the local ethical committee of Kagawa Medical University and standardized informed consent was obtained from each patient.

After resting for 30 min, the subjects were examined in the supine position. Maternal blood pressure and heart rate were recorded. At amniocentesis, a 20 ml sample of the amniotic fluid for fetal chromosome analysis and 3 ml for HGF and IGF-I measurements were obtained. Specimens (3 ml) were then centrifuged (1000 g for 10 min), and amniotic fluid was separated and stored at −80°C until assayed.

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.

IGF-I was measured by an immunoradiometric assay (IRMA) kit [IGF-I (Somatomedin-C) IRMA ‘Daichi’; Daiichi Pharmaceutical Co. Ltd, Tokyo, Japan]. The minimum detection limit of this assay for IGF-I was 0.03 ng/ml. The coefficients of intra-assay and inter-assay variation were 1.8 and 2.4% respectively.

All values were given as mean ± SD. Statistical analysis for comparison of gestational age at amniocentesis, birth age, birth weight, neonatal height, placental weight, and amniotic fluid HGF and IGF-I concentrations among the groups was performed using an analysis of variance and Newman–Keuls multiple comparison test. Statistical analysis for comparison of maternal age, and parity among the three groups was performed using a Kruskal–Wallis one-way analysis of variance by ranks and multiple comparisons. The Spearman rank test was used to assess correlation between amniotic fluid HGF or IGF-I concentrations and birth weight, height, or placental weight. P < 0.05 was considered significant.

### Results

Maternal age in AGA group (34.2 ± 5.5 years) was significantly younger than that in SGA (37.9 ± 3.0 years) and LGA (37.6 ± 3.3 years) groups respectively (P < 0.05). There were no significant differences for maternal height and weight, parity, and gestational age at amniocentesis among the groups. There were significant differences for birth age, birth weight, neonatal height, and placental weight among the groups respectively (P < 0.05) (Table I). HGF concentrations in SGA group were 16.9 ± 6.6 ng/ml, those in AGA group 16.7 ± 9.0 ng/ml, and those in LGA group 20.2 ± 14.8 ng/ml, but there were no significant differences for amniotic fluid HGF concentrations among the three groups (Table I). There was no correlation between amniotic fluid HGF concentrations and birth weight, height, or placental weight. IGF-I concentrations in the SGA group were 2.9 ± 0.8 ng/ml, those in the AGA group 2.8 ± 1.1 ng/ml, and those in the LGA group 2.6 ± 0.8 ng/ml, but there were no significant differences for amniotic fluid IGF-I concentrations among the three groups. There was also no correlation between amniotic fluid IGF-I concentrations and birth weight, height, or placental weight.

### Discussion

HGF has a potential to stimulate direct and random migration of endothelial cells (Morimoto et al., 1991), angiogenesis in the cornea (Bussolino et al., 1992), growth of gastric and intestinal cells (Fukamachi et al., 1994), and the growth and differentiation of multipotential and erythroid haemopoietic progenitor cells (Mizuno et al., 1993). 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 placenta cDNA library (Miyazawa et al., 1994). Schmidt et al.
(1995) and Uehara et al. (1995) demonstrated independently that HGF is essential for normal placentation in mice. Absence of either HGF or c-met seems to prevent the normal growth and development of the epithelially-derived labyrinthine trophoblast cells, leading to growth restriction and intrauterine death secondary to placental insufficiency (Somerset et al., 1997). Moreover, it has been shown that amnion secretes a considerable amount of HGF into amniotic fluid, which suggests the importance of HGF in relation to fetal growth (Horibe et al., 1995).

Before 20 weeks HGF concentrations in the amniotic fluid were relatively high, and they were significantly elevated between 20 and 29 weeks (Horibe et al., 1995). After 30 weeks, however, they dramatically decreased to concentrations lower than those before 20 weeks. These authors also demonstrated, by use of amnion organ culture, that HGF was released from amnion and the amount released from amnion during the second-trimester was considerably higher than that released from amnion of the third trimester. It was shown that amniotic fluid HGF concentrations at the second-trimester are negatively correlated with birth weight (Kurauchi et al., 1995). However, the number of observations (n = 20) reported in their study was very small, and the value of coefficient of determination was low. In this study, there were no significant differences for amniotic fluid HGF concentrations among the three groups (SGA, AGA, and LGA infant groups), and there was also no correlation between amniotic fluid IGFI concentrations and birth weight, height, or placental weight. Above mentioned studies on IGFI and IGF-I binding protein (Elgin et al., 1987; Ritovos et al., 1988; Wang et al., 1991) were all in-vitro studies, however, the results of this study were of an in-vivo investigation. Therefore, these differences in IGFI-I effect on fetal growth may be due to the difference in experimental methods.

In conclusion, the difference of HGF concentrations in the early second-trimester amniotic fluid does not predict fetal growth and placental size at birth. The presence of extremely high HGF concentrations in amniotic fluid may be involved in maturation of the fetal lung (Itakura et al., 1997) or digestive tract (Okamura et al., 1998), since these tissues become directly exposed through ingestion. Further study is needed to clarify the physiological role of amniotic fluid HGF as well as the origin of this factor.

References


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Received on March 16, 1999; accepted on July 1, 1999