Maternal Insulin Therapy Increases Fetal Endothelial Progenitor Cells during Diabetic Pregnancy

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ABSTRACT

Endothelial progenitor cells (EPCs) are bone marrow-derived cells involved in endothelial homeostasis and angiogenesis. Reduction and dysfunction of EPCs have been associated with the development of atherosclerosis and diabetic complications (1; 2).

Recent studies in human pregnancies suggest that mother’s EPCs are involved in the physiologic vascular remodelling of the systemic and utero-placental circulation (3; 4). Hyperglycemia induces dysfunction and apoptosis of EPCs (5), and this may impair the development or maturation of the utero-placental circulation, causing maladaptive responses during diabetic pregnancies. Indeed, EPCs has been shown to be dysregulated in pregnant women with diabetes (4).

On the other side, little is known on the effects of maternal factors on fetal EPCs. One study showed reduced cord blood EPCs in severe pre-eclampsia (6), while there are no data on diabetes. This study was undertaken to evaluate quantitative alterations of cord blood progenitor cells during pregnancies with diabetes mellitus.
RESEARCH DESIGN AND METHODS

We enrolled 24 non-diabetic and 17 diabetic pregnant women: 2 had pre-gravidic type 1 diabetes and 15 had gestational diabetes. Gestational diabetes was diagnosed according to the American Diabetes Association guidelines (7). Provided that informed consent was obtained from the mother, at time of delivery, 5 mL of umbilical vein cord blood were collected into heparin tubes for progenitor cell count. The following data about the mother were recorded: age, pre-gravidic BMI, weight increase, smoking habit, family history for cardiovascular disease, arterial blood pressure, and type of antidiabetic treatment. Before labour, blood samples were collected for the determination of plasma glucose, leukocyte count, fibrinogen levels, total cholesterol and triglycerides concentrations. After delivery, gestational age, neonatal weight and sex, and 1-5 min Apgar scores were recorded.

Progenitor cell count was performed using direct two- or three-color flow cytometry as previously described (8). Briefly, after red cell lysis, blood cells in the mononuclear cell gate were analyzed for the expression of surface antigen using FITC-conjugated anti-CD34 (Becton Dickinson), PE-conjugated anti-KDR (R&D Systems), and APC-conjugated anti-CD133 (Miltenyi Biotech) mAbs. CD34+, CD133+ and CD34+CD133+ cells were considered generic circulating progenitor cells, while CD34+KDR+, CD133+KDR+ and CD34+CD133+KDR+ cells were considered EPCs. Cell counts were expressed per 10^6 cytometric events.

Data are expressed as mean±SEM. Differences between two groups were assessed using Student’s t test and chi-square, where appropriate. Linear correlations between continuous variables were assessed using Pearson’s r coefficient, while multiple linear regression analyses were used to correct for potential confounders. SPSS ver 13.0 was used and statistical significance was accepted at p<0.05.

RESULTS

Diabetic pregnant women had a higher prevalence of arterial hypertension (29.4% vs 4%; p=0.02) and family history for cardiovascular disease (53% vs 16%; p=0.01) than non-diabetic women. There was a non-significant trend toward higher plasma glucose (92.5±6.9 vs 79.9±3.3 mg/dl; p=0.07), and higher birth weight (3453±91 vs 3214±77 g; p=0.06) in diabetic versus non-diabetic pregnancies, indicating an overall good metabolic control, as also witnessed by a near-normal HbA1c (6.1±0.2%). There were no significant differences in maternal age, pregravidic BMI, weight increase, smoking habit, gestational age, total cholesterol, triglycerides, leukocyte count, and fibrinogen levels, neonatal weight and sex, and Apgar scores. None of the participants had diabetic retinopathy at time of the study.

We found a trend toward reduction of cord blood CD34+, CD133+, CD34+CD133+, and CD34+KDR+ cell levels in diabetic versus non-diabetic pregnancies, which was nearly significant for CD34+ cell count (p=0.052). No significant differences were present in progenitor cell counts between type 1 and gestational diabetes. These data indicate that fetal EPCs are not significantly reduced in the presence of well-controlled maternal diabetes (figure 1A).

When diabetic women were divided according to the anti-diabetic treatment (diet alone versus diet plus insulin), we found higher levels of all progenitor cell phenotypes in women treated with insulin than in women on diet alone, which was largely significant for CD34+KDR+ cell count (172±50 vs 44±8; p=0.02; figure 1B). The association between maternal insulin therapy and higher fetal CD34+KDR+ cell count remained significant after correction for maternal age, hypertension, plasma glucose, family history
for cardiovascular disease, cholesterol and triglyceride values, and pregravidic BMI. These results suggest that insulin increases EPCs in the fetal circulation. In support of this hypothesis, we also show that the levels of fetal CD34^+KDR^+ EPCs were directly correlated with maternal daily insulin requirements (figure 1C). This correlation remained significant after correction for maternal age, hypertension, plasma glucose, and pregravidic BMI. On the contrary, no significant correlation was seen between fetal EPCs and maternal HbA1c.

CONCLUSIONS
EPCs are deputed to the maintenance of vascular integrity, and their level reflects cardiovascular health (1; 8). Moreover, EPC defects promote vascular disease, and emerging data indicate that dysregulation of maternal EPCs is associated with abnormalities of pregnancy. Given the comprehensive role played by EPCs in vascular development and homeostasis, the effects of maternal diabetes on fetal EPCs may have important consequences.

Herein, we show that well-controlled maternal diabetes is associated with a non-significant reduction of EPCs circulating in the fetal blood. Much interestingly, insulin therapy undertaken to achieve a near-normal glycemic control, was dose-dependently associated with higher fetal CD34^+KDR^+ EPC levels. It seems that the CD34^+KDR^+ phenotype was selectively modulated by maternal insulin therapy, Although there is considerable uncertainty on the exact EPC definition, this observation is in compliance with the notion that CD34^+KDR^+ may represent one of the most suited antigenic combinations to define EPCs in the clinical setting (9; 10).

Insulin has been previously shown to promote mobilization of EPC from the bone marrow to peripheral circulation in diabetic animals and in adult diabetic humans (11; 12). Our present data suggest that maternal insulin therapy increases EPCs in the fetal circulation. As insulin should not pass the placental barrier, an indirect effect on the fetal environment, possibly through the modulation of maternal and/or placental metabolism, must be postulated. In any case, this effect seems independent of glycemic control, because EPC levels and HbA1c were not closely correlated. Alternatively, insulin may induce placental VEGF, one potent stimulator of EPC mobilization and differentiation (2).

Currently, we don’t know whether EPCs are consistently modulated in the maternal as in the fetal circulation. Emerging data in animal models indicate that fetal EPCs pass the placental barrier and participate in maternal angiogenesis during pregnancy (13), while trafficking of EPCs in the opposite direction has not been substantiated. A simultaneous study of fetal and maternal EPCs may provide additional insights into the regulation of these cells during normal and diabetic pregnancy. Although the origin of fetal EPCs and the clinical significance of their increase remain unclear, it is possible that insulin therapy exerts protective effects on the fetal vascular system, through the stimulation of EPCs. Assessing whether this influences long-term neonatal outcomes is a major challenge to pursue.
REFERENCES

FIGURE LEGEND

Figure 1. A) Levels of cord blood (fetal) progenitor cells in the presence and in the absence of maternal diabetes. B) Levels of cord blood (fetal) progenitor cells in pregnant diabetic women divided according to the antidiabetic treatment (diet alone versus diet plus insulin). C) Significant direct linear correlation between fetal CD34⁺KDR⁺ cells and maternal daily insulin requirement.
FIGURE 1

**A**

Insulin stimulates fetal EPC

**B**

**C**

$r = 0.71$

$p = 0.05$