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Growth restricting effects of a single course of antenatal betamethasone treatment and the role of human placental lactogen

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ABSTRACT

Betamethasone (BET) is a widely used treatment for women who are at high risk of preterm delivery. In sheep, BET-induced growth restriction was found to be associated with reduced placenta lactogen (PL), a key regulator of fetal growth. We therefore hypothesized that also in humans a single course of BET administration is associated with a reduction of PL, associated with a deceleration in fetal growth.
Objective: To investigate effects of a single course of antenatal BET in humans on birth weight and PL.
Methods: Women exposed to BET (2 × 12 mg; $n = 44$) with normally grown fetuses between 23 + 5 and 34 + 0 wks (weeks + days of gestation) who delivered between 23 + 5 to 42 + 0 wks were compared to gestational age-matched controls ($n = 49$). Maternal gestational blood samples were obtained before, during and after BET treatment and at the time of birth.
Main outcome measures: BET effects on fetal anthropometrics, placental morphometry and placental PL-protein and maternal plasma levels.
Results: The mean duration of days between BET administration and birth was 52 days. BET treatment was associated with decreased birth weight (−18.2%), head circumference (−8.6%), body length (−6.0%), and placental width (−5.5%), as compared to controls. These changes were irrespective of possible maternal confounders (gestational age at birth, maternal age, maternal BMI gain during pregnancy, smoking etc.). However, neither PL-plasma levels within 48 h after BET treatment nor placental PL-protein levels and maternal plasma levels at birth were changed after BET treatment. In central regions of the placenta, BET treatment increased the circumference of syncytiotrophoblast nuclei by +4.7% and nucleus surface area by +9.4% compared to controls, but these changes were not related to placental PL-protein or maternal PL-plasma levels at birth.
Conclusion: A single course of BET treatment was accompanied with reduced fetal growth, but this growth restricting effect was not associated with altered placental or maternal plasma PL levels. Altered expression of PL appears not to be causal for BET-induced fetal growth restriction in the human.

1. Introduction

The administration of synthetic glucocorticoids (GC) is one of the most effective and important therapies in perinatal medicine. Treatment results in improved neonatal respiratory function and a decrease in morbidity and mortality [1]. However, repeated administration of GC and its effects on fetal development and long term health are the subject of ongoing controversy and uncertainty [2,3]. Inappropriate exposure of the fetus to GC has been proposed as a mechanism for 'fetal programming' with increased disease risk in later life [4]. Previous studies in humans have reported varying effects of antenatal glucocorticoids on birth weight and head circumference [5–7]. Growth restriction, altered

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hypothalamus–pituitary–adrenal function and insulin resistance in the offspring have been found in animal models [8–10], although the underlying mechanistic pathways are yet unclear. In human infants antenatal betamethasone (BET) exposure is associated with a measurable impairment of brain maturation, including reduced cortical folding as compared to controls [11]. BET reduced infants' hypothalamus–pituitary–adrenal (HPA) axis response up to 4–6 weeks after birth [12]. At the age of 3 and 6 years, repetitive antenatal BET administration was found to be associated with increased rates of aggressive and destructive, distractible and hyperkinetic behavior [13].

The placentas morphology and function itself can be altered by maternal glucocorticoid administration [14–22] and as the mediator between the mother and the fetus, it is likely that the placenta may play a role for respective developmental alterations [23].

The human placenta is covered by the syncytiotrophoblast, a unique terminally differentiated, multi-nucleated syncytium, which is sustained by continuous fusion of underlying cytotrophoblast cells [24]. The placental syncytiotrophoblast produces the growth hormone placental lactogen (PL), encoded by a gene cluster of five inter-related genes on chromosome 17 [25]. PL can be detected in the maternal circulation as early as 6 weeks of gestation with concentrations increasing towards term [26]. The role of PL in the regulation of maternal carbohydrate, lipid and protein metabolism and fetal growth has been previously demonstrated in several studies [26–29]. PL increases the glucose uptake into the maternal compartment, increases glycogen synthesis, glucose oxidation and insulin secretion and has maternal diabetogenic effects. PL induces maternal lipolysis and proteolysis and increases nutrient partitioning to the fetus [29].

PL may have a direct role in the regulation of fetal growth, but its effects are more likely indirect, through alterations in the maternal environment, maternal placental nutrient transfer to the fetus or through stimulating the release of fetal insulin-like-growth factor (IGF) release [30]. The synthesis of PL has been suggested to be unaffected by circadian variation [31,32]. Maternal fasting and hypoglycemia however, increase maternal PL-plasma levels, while hyperglycemia results in a reduction in PL-levels [33–35]. Pregnancies with placental insufficiency and intrauterine growth restriction (IUGR) have been associated with decreased PL-plasma levels in the maternal circulation [36].

Fetal growth restriction has been observed in pregnancies with GC-exposure [5–7,37]. We have previously shown in sheep, that repeated maternal intramuscular injections of betamethasone (BET) in the last third of gestation decreased the number of binucleate cells (BNC), which are the source of PL in sheep, reduced ovine placental PL-protein and reduced circulating ovine PL-levels in the mother and the fetus [14]. We found that BNC numbers, placental PL-protein levels and circulating maternal and fetal PL-levels were positively associated with fetal weight [14]. Further, fetal cortisol infusion in sheep during late gestation decreased the number of BNCs in the fetal trophectoderm [38]. Based upon these observations, we hypothesized that the growth restricting effects of GC could be mediated, at least in part, by effects on PL secretion and subsequent actions on maternal-placental metabolic function, maternal and fetal growth factor secretion and/or function. We therefore hypothesized in a translational sense, that in the human changes of PL may also be involved in glucocorticoid-induced fetal growth restriction. Consequently, we investigated the short- and long-term effects of a single course of intramuscular maternal BET administration on placental size, as well as placental and maternal plasma PL-levels in human pregnancy and potential relationships with neonatal anthropometrics (birth weight, head circumference, body length).

2. Materials and methods

All experimental procedures were approved by the local Ethics Committee of the Charité-University Medicine, Berlin, Germany (EA2-149-07).

2.1. Study design

Pregnant women exhibiting signs of preterm delivery were prospectively recruited into the study. Inclusion criteria were: symptomatic contractions with shortening of the cervical length, cases with prolapsed fetal membranes, preterm premature rupture of membranes (pPROM) or vaginal bleeding. Exclusion criteria were: multiple gestations, severe malformations noted at birth, pregnancies in which a different GC than BET was given for lung maturation, treatment at gestational ages other than 23 + 5 to 34 + 0 wks (weeks + days of gestation), pregnancies complicated by hypertension, preeclampsia, eclampsia, liver disease and/or diabetes.

Tocolytic treatment according to local guidelines with nifedipine or fenoterol was given and a single course of antenatal BET (2 × 12 mg 24 h apart) was maternally administered between 23 + 5 and 34 + 0 wks in normally grown fetuses to improve fetal lung maturation, according to the actual recommendations [39]. Fetal weight before BET treatment was estimated by ultrasound using the Hadlock formula [40]. Fetuses with estimated fetal weight (EFW) below the 10th centile at the time point of BET treatment were defined as small for gestational age (SGA) and were excluded from the study [41]. Estimated date of delivery was corrected by early prenatal ultrasound (crown-rump length) when the difference between due date by last period and early sonography was more than 7 days.

According to our previous findings in sheep, with a reduction in ovine PL plasma levels of up to 40% after BET administration [14], a sample size of $n = 44$ patients for each group was calculated (power = 0.9, alpha = 0.05, sd1 = 4.0, sd2 = 2.5, $r = 0.6$) for this study. BET exposed women ($n = 44$) who gave birth between 24 + 0 and 42 + 0 wks were compared to gestational age-matched controls ($n = 49$). In controls, the mean gestational age at delivery was 267 days (range: 222–291), in BET 249 days (range: 177 to 293; $p < 0.05$). The effects of BET on neonatal anthropometrics (fetal weight in gram, body length in cm, head circumference in cm, ponderal index (calculated by 100 × birth weight (kg)/body length (cm)^3), umbilical cord blood gases with umbilical artery (UApH) and vein pH (UVpH), base excess, 1-, 5- and 10-min Apgar scores), and placental parameters (placenta weight in gram, placenta width in cm, placenta thickness in cm, placenta surface area cm^2) were analyzed.

Four maternal plasma samples were collected from each pregnant women: one sample was collected just before the first dose of 12 mg BET was given, the second sample was collected 24 h later just before the second dose of 12 mg BET was given. The third sample was collected 48 h after the first dose of BET treatment. The forth sample of maternal plasma was collected during delivery at a stage of 4–5 cm cervical dilatation. Whole central and peripheral placenta biopsies were collected according to a systematic and uniform random sampling protocol as described previously [42]. Briefly, placenta biopsies were collected with an overlaying grid placed on top of the placenta. The position of the first tissue section was chosen randomly, the other biopsies were selected according to the pre-determined patterns of the grid within the peripheral and central zone of the placenta. Tissue sections were snap frozen in liquid nitrogen and stored at −80 °C until further use.

2.2. Immunohistochemical quantification of PL-positive stained syncytiotrophoblast

Immunohistochemical detection of PL-positive syncytiotrophoblast was performed on 5 μm paraffin embedded placental sections. Cross sections were taken from the central region and margin of the placenta. A polyclonal rabbit antibody against human PL (1:600 dilution, number: ab15554; ABCAM, Cambridge Science park, Cambridge, UK) was used with avidin-biotin-peroxidase reagents (Elite Vectastain ABC Kit, Vector Laboratories, Linaris GmbH, Wertheim-Bettingen, Germany) as described previously [43]. Placental tissue sections from both the treatment and control group were processed simultaneously to allow direct comparison between assays. Each assay included one tissue section for an internal control (38 + 4 wks). Negative controls were as follows: (i) the primary PL-antibody was substituted either by an antibody dilution buffer or by nonimmune rabbit serum (1:600 dilution), (ii) the peroxidase labeled secondary link antibody (goat anti-rabbit immunoglobulin) was substituted with PBS (pH 7.5) wash buffer, and (iii) the slide section was only incubated with PBS (pH 7.5) diluent before the addition of the substrate-chromogen solution.

2.3. Image analysis

Semi-quantitative analyzes were performed using computerized image analysis (KS 400 3.0, Carl Zeiss, MicroImaging GmbH, Berlin, Germany). The effect of BET on placenta growth within distinct compartments was monitored as density of syncytiotrophoblast nuclei (number/mm^2) [44], and syncytiotrophoblast function was estimated by measuring syncytiotrophoblast nucleus circumference (μm), from which surface area (μm^2) was calculated [45,46]. Additionally, cytoplasmic PL-staining intensity was determined. Parameters were measured in a semi-automated,