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Sex-specific and lasting effects of a single course of antenatal betamethasone treatment on human placental 11β-HSD2

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ABSTRACT

Introduction: We have previously shown that even a single course of antenatal betamethasone (BET) as an inductor for lung maturity reduces birth weight and head circumference. Moreover, animal studies link BET administration to alterations of the hypothalamic-pituitary-adrenal-gland-axis (HPA). The unhindered development of the fetal HPA axis is dependent on the function and activity of 11β-hydroxysteroiddehydrogenase type 2 (11β-HSD2), a transplacental cortisol barrier. Therefore, we investigated the effects of BET on this transplacental barrier and fetal growth.

Methods: Pregnant women treated with a single course of BET between 23 + 5 to 34 + 0 weeks of gestation were compared to gestational-age-matched controls. Placental size and neonatal anthropometrics were taken. Cortisol and ACTH levels were measured in maternal and umbilical cord blood samples. Placental 11β-hydroxysteroiddehydrogenase type 1 (11β-HSD1) protein levels and 11β-HSD2 protein and activity levels were determined. Parameters were analyzed independent of sex, and in subgroups divided by gender and gestational age.

Results: In term born females, BET administration was associated with reduced head circumference and decreased 11β-HSD2 protein levels and enzyme activity. Males treated with BET, especially those born prematurely, showed increased 11β-HSD2 protein levels.

Conclusion: A single course of BET alters placental glucocorticoid metabolism in a sex-specific manner. Decreased 11β-HSD2 levels in term born females may lead to an increased placental transfer of maternal cortisol and therefore result in a reduced head circumference and a higher risk for altered stress response in adulthood. Further research is needed to conclude the significance of increased 11β-HSD2 levels in males.

1. Introduction

Chronic exposure of the fetus to high levels of glucocorticoids has been discussed as a mechanism for fetal programming of postnatal disease risk [1–3]. We have previously shown that even a single course of antenatal Betamethasone (BET) to mature fetal lungs reduces birth weight and head circumference in humans [4–6]. We, and others, have also shown that in experimental models, antenatal BET exposure results in an altered hypothalamic-pituitary-adrenal-gland (HPA) axis responsiveness to postnatal stress and is often associated with metabolic dysfunction [7–14].

The unhindered development of the fetal HPA axis is dependent on a placental cortisol barrier largely regulated by a placental enzyme, 11beta-hydroxysteroiddehydrogenase type 2 (11β-HSD2) [15–17]. 11β-HSD2 metabolizes cortisol to cortisone, where only 10–20% of maternally derived cortisol is permitted to pass through the placenta to the fetus [15,18]. Any disturbance in this placental barrier leads not only to disrupted development of the fetal HPA axis, but is also associated with fetal hypertension, hyperinsulinemia, and impaired fetal growth [2,15,19]. Therefore, it has been proposed that 11β-HSD2 plays

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a pivotal role in mediating the observed short- and long-term effects of a single course of antenatal BET [20–22].

Animal studies report upregulation of 11β-HSD2 mRNA in rat and baboon placentas and both up- and downregulation in ovine placentas after antenatal glucocorticoids [23–26]. In the human placenta, Johnstone et al. found no significant effect of a single course of antenatal BET on 11β-HSD2 protein levels and enzyme activity in preterm placental tissue [27]. However, this study was primarily designed to analyze the effect of chorioamnionitis and only included 12 samples. Moreover, in this study and the aforementioned animal studies birth occurred prematurely shortly after BET administration [23–27]. Considering that 52% of women treated with antenatal glucocorticoids deliver more than a week after BET administration, more investigation is needed into the potential lasting effect of antenatal glucocorticoids on 11β-HSD2 in the human placenta [28].

Furthermore, Johnstone et al. did not conduct sex-specific analyses, despite the known sex-dependent placental adaptation to adverse intrauterine environments [29,30]. Females born up to 72 h after BET administration showed an increased 11β-HSD2 activity compared to male counterparts and females born 72 h after BET [31], however there was no control group of untreated neonates. A sex-dependent effect of BET has been also demonstrated in children aged 6–11 years old, where only females demonstrated significantly increased stress reactivity after antenatal BET [32].

In contrast to 11β-HSD2, the main function of 11β-hydroxysteroiddehydrogenase type 1 (11β-HSD1), is the reduction of cortisone to cortisol [15,33]. Li et al. report an upregulation of 11β-HSD1 expression by cortisol in human chorionic trophoblasts cultures [34]. However, it is unknown if antenatal BET has the same effect *in vivo*.

In the present study, we hypothesized that a single course of BET would reduce levels of placental 11β-HSD2, allowing transplacental passage of maternal cortisol, inducing fetal growth restriction and a suppression of the developing fetal HPA axis. Because previously published studies suggest an increased vulnerability of females, we hypothesized this BET effect to be more pronounced in females than in males [30,32].

2. Material and methods

This study was approved by the Ethics Committee of the Charité - Universitätsmedizin, Berlin, Germany (EA2-149-07).

2.1. Study design

Pregnant women exposed to a single course of antenatal BET for lung maturation (2 × 12 mg intramuscular; n = 86) between 23 + 5 to 34 + 0 wks (weeks + days of gestation) were recruited prospectively before birth and compared to a gestational-age-matched control group that received no antenatal BET (n = 92). Exclusion criteria were: multiple pregnancy, severe fetal malformations, repeated courses of BET or administration of glucocorticoids other than BET, estimated weight below the 5th and exceeding the 95th percentile [35], pregnancies complicated by diabetes, hypertension, preeclampsia and eclampsia. All participants were de-identified, thus blinding their treatment status to experimenters.

Maternal venous blood samples were taken at delivery at approximately 4–5 cm cervical dilation. After delivery, venous cord blood samples were obtained and frozen at −80 °C in vials with low bind properties. Placental measures (placental weight, width, length, thickness and surface area) were recorded. Whole placental biopsies were taken as previously described using a systematic and uniform random sampling protocol [5,36]. Due to the possible regional differences in gene expression and vascularization within the placenta, biopsies were taken from both peripheral and central areas [37]. Samples were snap frozen in liquid nitrogen and stored at −80 °C. The remaining placental tissue was placed in formalin and sent for pathological evaluation.

Neonatal anthropometrics were measured in the context of postnatal assessment by trained midwifes using the same measuring tape and accordingly the same weighing scale. Neonatal outcome parameters (APGAR scores after 1-, 5- and 10 min, base excess, umbilical artery pH (UApH) and umbilical vein pH (UVpH)) were recorded.

2.2. Quantification of ACTH and cortisol plasma levels

Plasma ACTH and cortisol levels in maternal and umbilical venous samples were analyzed using a commercially available ELISA kit according to the manufacturer's protocol. For the ACTH ELISA (EIA-3647, DRG Instruments GmbH, Marburg, Germany), we prediluted plasma samples 1:2. Samples beyond our standard range (Fig. S1 A) were diluted further or measured undiluted, the minimum detection limit was 0.22 pg/ml. The intra-assay coefficient of variability was 3.5% and the inter-assay coefficient of variability was 17.1%.

For cortisol ELISA (MS E − 5000, Labor Diagnostika Nord GmbH & Co. KG, Nordhorn, Germany), we prediluted plasma samples 1:6. Samples beyond our standard range (Fig. S1 B) were diluted further and or measured undiluted, the minimum detection limit was 0.4 µg/dl. The intra-assay coefficient of variability was 2.2% and the inter-assay coefficient of variability was 9.6%.

2.3. Quantification of placental 11β-HSD1 and 11β-HSD2 protein levels

Placental 11β-HSD1 and 11β-HSD2 protein levels were quantified by Western blotting. The polyclonal goat anti-human 11β-HSD1 antibody (LOT P1E270509, AP16309PU-N, Acris Antibodies, Inc., San Diego, USA) and the polyclonal sheep anti-human 11β-HSD2 antibody (LOT 318564, The Binding Site Group Ltd, Birmingham, UK) were used and tested beforehand in immunohistochemistry on 5 µm paraffin embedded placental sections, both in a dilution of 1:250 [27,38,39] (Fig. S2).

Briefly, membranes were first incubated with the 11β-HSD1 antibody at a dilution of 1:1000 in PBS-Tween overnight. The 11β-HSD1 34 kDa-bands were then analyzed with the ChemiDoc MP Imaging System (Bio-Rad Laboratories GmbH, Munich, Germany; Fig. S3 A). Afterwards, the blots were incubated overnight with the 11β-HSD2 antibody at a dilution of 1:1000 in 5% skim milk powder and analyzed on the next day (Fig. S3 B). Each blot was repeated at least three times and included an internal control sample that controlled for inter-blot variability. Protein results are expressed as ratio of 11β-HSD1, or 11β-HSD2, to β-Actin (1:80000, LOT GR67149-2, Abcam plc, Cambridge, UK) and expressed as relative optical density units (ROD).

2.4. Placental 11β-HSD2 activity assay

Considering the significantly decreased peripheral 11β-HSD2 protein in term born females treated with BET, we decided to measure placental 11β-HSD2 activity rate in this subset (BET n = 21, controls n = 33) to confirm this result. The activity assay was adapted from previously published protocols [40,41].

Briefly, 75 µg/ml protein of each sample was incubated in triplicate in a sodium phosphate buffer with 1 mM NAD (AE11, Carl Roth GmbH, Karlsruhe, Germany), 5 µM cold cortisol (SLBH9234V, Sigma-Aldrich, Saint Louis, USA) and 0.3 µCi [1,2,6,7-3H]-cortisol (LOT, 2077604, PerkinElmer, Inc., Boston, USA) for 30 min at 37 °C in a shaking water bath. Reaction was stopped by adding 1.5 ml ice-cold ethyl acetate to each sample. After freezing the samples for 15 min at −80 °C, the organic phase was removed into a vial and dried overnight in a vacuum concentrator (SC110 Speedvac concentrator, Savant Instruments, Inc., Holbrook, USA). On the next day steroids were reconstituted with 100 µl ethyl acetate. After adding 10 µl of a 10 mM solution of cold cortisol and 10 µl of a 10 mM solution of cortisone the vials were rotated for 40 min on a rotator. 25 µl of each sample were pipetted onto a thin layer chromatography plate (1167170001, Merck KGaA,