Glucocorticoids in Pregnancy

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Abstract: The fetus may be exposed to increased endogenous or synthetic glucocorticoid (GS) exposure in late gestation. Approximately 7% of pregnant women in Europe and North America are treated with synthetic GSs to promote lung maturation in fetuses at risk of preterm delivery. Maternal steroid treatment before preterm delivery is one of the best documented and most cost effective life saving treatments in prenatal medicine but, in certain circumstances, the price of accelerated lung maturity may be loss of brain cells, increased neurodevelopmental disability, intra-uterine growth restriction (IUGR), and an increased risk of preterm delivery, of programming of post-natal hypertension, and of increased post-natal activity in the hypothalamo-pituitary-adrenal (HPA) axis. Placental 11β-hydroxysteroid dehydrogenase type 2 (11β-HSD2) is the key enzyme which protects the fetus from overexposure to GSs by their oxidation into inactive derivates. We review the evidence for the metabolism of GSs during pregnancy and how endogenous and synthetic GSs cause other changes in the placenta which affect fetal development.

Keywords: Antenatal exposure, glucocorticoids, 11β-hydroxysteroid dehydrogenase, metabolism, placental transport, pregnancy.

INTRODUCTION

Antenatal glucocorticoids (GSs) were first introduced into perinatal practice in 1972 by Liggins and Howie who showed that their use could reduce the incidence of respiratory distress syndrome in premature infants [1]. Since then, the administration of GSs to pregnant women at risk of preterm delivery has become an established intervention.

Glucocorticoid hormones regulate many of the processes required for successful embryoimplantation, as well as for the subsequent growth and development of the fetus and placenta. They have been postulated to have a role in the stress response to delivery, fetal development and maturation, uteroplacental adherence, and the initiation of parturition.

GSs are essential for the development and maturation of fetal organs before delivery, and late pregnancy is characterized by a rise in cortisol levels, which parallels the increased maturity of fetal organs [2].

Proper GS exposure in utero is vital for normal fetal organ maturation, but excess GSs are detrimental to fetal growth and can even predispose the individuals to the high risk of having certain diseases in adulthood, such as hypertension, diabetes and strokes [3-5]. It has been proposed that the fetus is protected from maternal GS levels 10 times higher by the placental enzyme 11β-HSD2, which converts biologically active cortisol to inactive cortisone requiring nicotinamide adenine dinucleotide (NAD) as its cofactor [6, 7]. This placental GS barrier was functional not only at term, but also at the early and mid gestational ages [8].

ANTENATAL GLUCOCORTICOID THERAPY

GSs are used for a variety of common conditions in women of reproductive age (i.e. allergy, asthma, collagen vascular diseases, inflammatory bowel disease, etc.). Prednisone, prednisolone, and methylprednisolone have minimal placental transfer and they are the drugs of choice during pregnancy. Fluorinated GSs such as dexamethasone and betamethasone easily cross the placenta and should not be used unless there is intent to treat the fetus [9].

Gur et al. [10] prospectively collected and followed up 311 pregnancies exposed to systemic GSs at least in the first trimester of pregnancy. Most women (65.4%) were exposed only during the first trimester, 8.4% were treated during the first and second trimesters, while 26.2% were treated throughout the pregnancy. The majority of the women in this cohort were exposed to prednisone (70.0%). Other GSs were: betametasonse, cortisone, dexamethasone, fluocortolone, methylprednisolone, prednisolone, hydrocortisone and triamcinolone. The most common indications for treatment were allergy and asthma; the rest of group was treated because of inflammatory bowel disease, systemic lupus erythematosus, rheumatoid arthritis, arthritis, recurrent fetal loss, antiphospholipid antibody syndrome, erythema nodosum and transplantation. The majority of patients in this cohort (86.2%) used the medication orally, while 13.8% used it by intravenous or intramuscular routes [10].

Approximately 7% of pregnant women in Europe and North America are treated with synthetic GSs to promote lung maturation in fetuses at risk of preterm delivery. Betamethasone administration has confirmed the effectiveness of GSs in maturing the fetal lungs because it lowers the incidence of neonatal respiratory distress syndrome and its associated mortality [1]. GS treatment has been shown to result in an increase in the ratio of lecithin to sphingomyelin in
amniotic fluid, an indicator of fetal lung development and surfactant synthesis [11]. Betamethasone (two 12-mg intramuscular doses 24 hours apart) or dexamethasone (6 mg intramuscularly every 12 hours for four doses) administered to women with preterm delivery between 24 and 34 weeks (wks) gestation, results in a decreased incidence of neonatal mortality, respiratory distress syndrome, and intraventricular hemorrhage [12]. Antenatal GSs also benefit patients with preterm premature rupture of membranes (PPROM) and those with hypertensive syndromes.

Repeated courses of GSs are not recommended [13]. Repeated courses of betamethasone were associated with a 4% reduction in head circumference and a 9% reduction in birth weight in preterm infants born before 33 wks gestation, compared with single courses of betamethasone [14].

**GLUCOCORTICOIDS DURING PREGNANCY**

GSs and progesterone are steroid hormones that can diffuse easily across the plasma membrane. Once in the cytosol, they can bind to their respective cytoplasmic receptors. Ligand-bound receptors can dimerize and translocate to the nucleus of the cell to act as transcription factors to promote or prevent the transcription of target genes. Alternatively, these ligand-bound receptors can bind as single polypeptides to other transcription factors, interfering with their actions to promote changes in cellular activity. Although certain cells are associated with the primary production of specific steroid hormones, all steroid hormones are formed from a common precursor (i.e. cholesterol), and any cell with the appropriate enzymes can potentially produce specific steroid hormones.

Cortisol can activate both the classical glucocorticoid-responsive receptor (GR) as well as the mineralocorticoid receptor (MR). The affinity of the MR for corticosterone and for cortisol is similar to that for aldosterone. GR and MR have been demonstrated to be functionally expressed in the human trophoblast. Cortisol signals depend upon receptor availability and the local concentration of this steroid hormone [15].

The concentrations of cortisol in the maternal plasma are three- to fourfold higher than those in the fetal circulation. For much of pregnancy, these relative levels are maintained, in part because of the functional state of the fetal HPA axis, and in part because of a transplacental barrier to the transfer of cortisol from mother to fetus. In the placenta, 11β-HSD2 protects the fetus from the potentially harmful effects of endogenous maternal GSs. Several studies have described a relationship between the reduced activity of 11β-HSD2 and reduced birth weight or IUGR [16, 17], if placental (NADP+/NADPH) [29].

**PHYSIOLOGY, EXPRESSION, LOCALIZATION AND REGULATION OF 11B-HSD ISOFORMS**

Studies by Cope and Black [27] showed that the biological activity of any GSs relates, in part, to the presence of a hydroxyl group at position C11 of the steroid structure, and the inactivation of this group to a C-11 oxo group inactivates the steroid. Amelung and colleagues discovered in 1953 that the enzymatic interconversion of cortisol in humans, and corticosterone in the rodent was performed by the 11β-hydroxysteroid dehydrogenase 11β-HSD enzyme [28].

Two isoforms of 11β-HSD have been cloned and characterized in humans which interconvert GSs with their 11-keto metabolites (Fig. (1)). Both cloned isoforms of 11β-HSD are members of the short-chain alcohol dehydrogenase superfamily of enzymes. The biochemical properties of the two cloned 11β-HSD enzymes are summarized in Table 1. 11β-HSD2 has an absolute requirement for the oxidized form of NAD+ as an enzyme cofactor, whereas 11β-HSD1 preferentially utilizes nicotinamide adenine dinucleotide phosphate (NADP+/NADPH) [29].

The principal role of 11β-HSD1 is to regenerate cortisol or corticosterone to maximize activation of the GRs [30]. However, whereas 11β-HSD1 acts predominantly as a ketosteroid reductase in intact cells, this enzyme is inherently bidirectional; in cell homogenates provided with NADP+, 11β-HSD1 can inactivate GSs, albeit with a low affinity (Table 1). In the steroidogenic cells of the testis, ovary and placenta, the activity of NADPH-dependent cytochrome P450 (P450)
enzymes, required for the biosynthesis of steroids, favors the oxidation of NADPH to NADP+, thus promoting the oxidative activity of 11β-HSD1 [29].

Chan et al. [31] suggested that localized expression of 11β-HSD1 is likely to be a key determinant of GS responses in decidua, particularly towards the end of gestation. 11β-HSD1 plays a significant role in defining the bioactivity of GSs in the fetal-placental unit. A possible role of 11β-HSD1 in late gestation may be to regulate the apoptosis of specific decidual leukocyte populations, such as uterine natural killer cells, T cells, or macrophages [31].
Unlike 11β-HSD1, 11β-HSD2 has been localized to mineralocorticoid target cells in kidney, colon and parotid glands, as well as to the pancreas and placenta. With physiological substrates, 11β-HSD2 acts exclusively as an NAD+-dependent, high affinity 11β-dehydrogenase (Table 1) [29]. In the distal nephron, in adult life, 11β-HSD2 protects MRs from activation by corticosterone [32]. Its deficiency leads to apparent mineralocorticoid excess in which GSs illicitly activate MR, causing sodium retention, hypertension and hypokalemia. Although 11β-HSD2 is barely expressed in the adult brain, the enzyme is highly expressed in the developing central nervous system (CNS), until the end of mid-gestation in rats, mice and humans [33]. Holmes et al. [34] suggested that 11β-HSD2 expression in the early postnatal brain acts to protect the developing nervous system against the deleterious consequences of GS overexposure, which may otherwise result in longlasting behavioral and functional defects.

Type 1 enzyme (11β-HSD1) is expressed in the decidua, chorion, amnion and in the endothelial cells. Type 2 11β-HSD, the main functional isozyme in the placental syncytiotrophoblast, is expressed in both the decidua and in the syncytiotrophoblast [35]. In the baboon placenta, 11β-HSD1 and 11β-HSD2 are co-expressed in the syncytiotrophoblast [36], and in the mouse placenta both cloned isoforms of 11β-HSD are co-expressed with GRs in the labyrinthine zone in the later stages of gestation [35]. In all species, this localization of 11β-HSD2 at the materno-fetal interface reflects the role for 11β-HSD2 as a mechanism to limit transfer of GSs between the maternal and fetal circulations.

Circulating levels of GSs increase as human gestation progresses, with the highest levels present at parturition. At different stages of gestation, the balance between the reductase activity of 11β-HSD1 and the oxidative activity of 11β-HSD2 in the placenta changes in a species-specific manner. In ewes and guinea-pigs, placental 11β-HSD activity decreases as pregnancy progresses, whereas in human, baboon, pig and rat placentas, 11β-HSD activity increases in the later stages of gestation [29]. Giannopoulos et al. [8] examined placental 11β-HSD activity and found that type 2 activity predominated and that this activity decreased from early (8–12 wks) to late (38–40 wks) gestation. Similarly, Blasco et al. [37] described a decrease in placental 11β-HSD2 activity from early to late gestation. Studies have shown an increase in the 11β-HSD1 conversion of cortisone to cortisol in the fetal membranes with advancing gestational age [38]. These studies suggest that the barrier function of 11β-HSD, although effective throughout pregnancy, may decrease with increasing gestation. More recent studies have described an increase in 11β-HSD2 activity [6] and mRNA abundance [39] in the placenta from mid- to late-gestation.

The expression and activities of placental 11β-HSD isoforms is hormonally regulated. In placental and chorionic trophoblasts in humans, both progesterone and oestradiol selectively inhibit the oxidative activity of 11β-HSD2 without affecting 11β-HSD1 reductase activity [40]. Expression or activity of this high affinity 11β-HSD is also inhibited in human term placental trophoblasts by noradrenaline [41], nitric oxide [42], prostaglandins and leukotriene B4 [43]. In contrast to these suppressive actions, placental 11β-HSD2 is selectively upregulated by cyclic adenosine monophosphate (cAMP) [40]. Recent studies have demonstrated that human chorionic gonadotropin (hCG) secreted by the syncytiotrophoblasts might act in a paracrine or autocrine way to maintain the 11β-HSD2 expression and in the placental GS barrier, via the cyclic adenosine monophosphate/protein kinase A (cAMP/PKA) pathway. The enhancement of hCG production by cortisol may account, at least in part, for the induction of 11β-HSD2 by cortisol in the placenta [44].

The placental hypoxia associated with preeclampsia alters the transplacental passage of GSs from mother to fetus, and the local balance of GS regeneration from 11-ketosteroids [45]. Some studies have shown that hypoxia represses expression of 11β-HSD2 (without affecting 11β-HSD1) in term human trophoblasts and villous explants [45, 46].

Within the human placenta, there is also substantial metabolism of cortisol (and cortisone) by 3β-reductase, 3αβ-HSD and 20β-HSD enzymes to form inactive tetrahydro- and hexahydro-steroid metabolites [47].

The midgestation human fetus (16–19 wk) contains 11β-HSD2 mRNA and activity, but not 11β-HSD1, in the kidney, lung [48], gonad, liver, adrenal [49], and colon [50]. The presence of placental 11β-HSD2, high levels of 11β-HSD2 activity in fetal tissues, and the absence of 11β-HSD1 in the fetus all contribute to a predominance of cortisone over cortisol in the fetal circulation [51]. The presence of the 11β-HSD2 enzyme in the fetal tissues may serve to locally control the positive and negative effects of GSs on the fetus.

The placenta has also the capacity for the synthesis and metabolism of steroids and protein hormones. In human pregnancy, the placenta is a major site of steroid biosynthesis. It utilizes cholesterol from the low density lipoproteins (LDL) fraction in maternal blood for the production of progesterone. The placenta expresses abundant P450 side chain cleavage and 3β-HSD activity, and the major rate limiting step in its ability to synthesize progesterone appears to be adequate uteroplacental perfusion and the provision of steroids and protein precursors.

The human placenta, however, lacks the enzyme P450 C17. It is therefore unable to utilize C21steroids such as pregnenolone or progesterone as substrates for the production of C19 androgens, precursors for oestrogen biosynthesis. However, the human placenta is able to use C19 steroids of maternal and fetal adrenal origin for oestrogen biosynthesis.

**GLUCOCORTICOID THERAPY-ADVERSE EFFECTS**

A growing body of evidence indicates that increased exposure of the fetus to GSs in mid- to late-pregnancy may result in an adverse outcome, which includes [47]:

- Intra-uterine growth restriction
- Increased risk of preterm labor
- Programming of post-natal hypertension
- Programming of increased post-natal activity in the HPA axis
- Effects on fetal brain development, associated with alterations in pre-natal and post-natal behavior.
Antenatal dexamethasone treatment has been associated with a reduction in birth weight, by as much as 161 g in infants delivered between 30 and 32 wks [52]. Holmes et al. have shown that loss of 11β-HSD2 activity results in an early life exposure to high maternal GSs, resulting in low birth weight and a programmed behavioral phenotype of increased anxiety [5]. The effects of GSs on fetal growth may also be mediated by changes in insulin-like growth factor 1 (IGF-I). In pregnant rats, treatment with betamethasone or dexamethasone decreased maternal plasma IGF-I, which was related to a reduced liver-to-body weight ratio [53]. In human preterm infants treated with dexamethasone postnatally, there was evidence that the growth-suppressing effects of dexamethasone may be mediated by suppression of the IGF axis [54].

Few longitudinal studies have been performed in children whose mothers received repeated courses of prenatal GSs. In one study, three or more courses were associated with increased rates of hyperactivity later in childhood [4]. A long-term follow-up study revealed no effects of a single course of prenatal betamethasone on the intellectual capacity, gender development, sex-specific cognitive function, and psychoneuroticism at 20 years of age [3]. In one comprehensive follow-up study of children treated with dexamethasone in suspected cases of congenital adrenal hyperplasia, no adverse effects of prolonged GS exposure on cognitive and developmental outcomes at 12 years of age were identified [55].

The upregulation of 11β-HSD2 enzyme expression during the differentiation of cytotrophoblasts into syncytiotrophoblasts is compromised during hypoxia [56]. Likewise, compromised 11β-HSD2 expression and activity ex vivo and in vitro were detected in human placenta and villous explants, if oxygen delivery was limited [15, 45]. Kajantie et al. concluded that, in small preterm infants, reduced placental 11β-HSD2 function is associated with low relative birth weight and severe fetal distress [16]. Also, in placental tissue of small-for-gestational-age (SGA) neonates, 11β-HSD2 and 11β-HSD1 gene expression was found to be reduced [57]. Dy et al. [58] suggested that not only placental, but also fetal 11β-HSD2 activity, may be compromised in idiopathic IUGR. Although there is strong evidence that placental 11β-HSD2 is reduced in pregnancies complicated with IUGR, the underlying molecular mechanisms remains elusive.

Reduced 11β-HSD2 mRNA expression has been identified in placental tissue matched for gestational age and controlled for mode of delivery in preeclampsia when compared to normal pregnancies [59, 60]. Pregnanacies complicated by maternal hypertension and preeclampsia have been associated with compromised 11β-HSD2 activity and/or availability. 11β-HSD2 activity in preeclampsia correlates with factors associated with increased vasoconstriction, such as an increased angiotensin II receptor subtype 1 expression. Numerous signals such as proinflammatory cytokines, known to be present and/or elevated in preeclampsia, regulate 11β-HSD2 activity. An insufficient trophoblast invasion with the resulting hypoxemia seems to critically reduce the available 11β-HSD2 activity [15].

Although there are reports that GS use in pregnancy is associated with cleft palates in rabbits and mice, the occurrence of such congenital anomalies is rare in humans and there is no solid evidence that these are more common than the background incidence of congenital anomalies in normal pregnancies [10].

**PRENATAL STRESS**

Recent human studies have shown that a wide variety of prenatal stressors, from anxiety and partner relationship problems, to natural disasters, increase the risk of a diverse range of adverse neurodevelopmental outcomes in the child. However, many questions remain about the underlying processes. Much of the research, based on animal studies, has focussed on the maternal HPA axis, with mixed results. Maternal stress or anxiety during pregnancy has been found to be weakly associated with raised maternal cortisol, if at all. The placenta may be a more promising programming vector, because it controls fetal exposure to the maternal environment. Animal studies indicate that prenatal stress can affect the activity of the placental barrier enzyme 11β-HSD2, which metabolises cortisol [61].

Maternal stress leads to numerous cardiovascular and endocrine changes in the mother, including increases in the plasma adrenocorticotropic hormone (ACTH), β-endorphin, GS and catecholamine concentrations. The placenta forms a structural and biochemical barrier to many of these maternal factors, though a number will still enter the fetus. GSs have become a primary candidate for programming the fetal HPA axis during prenatal stress. Maternal and fetal plasma corticosterone are significantly elevated after maternal stress in rats [62]. In the guinea pig, cortisol concentrations in the maternal plasma are 10-fold those in the fetus; prenatal stress results in significant increases in plasma GS concentrations in both mother and fetus [63]. Interestingly, an earlier study demonstrated that greater GS transfer occurs across the placenta of female, compared to male, fetuses [64].

In rat offspring, prenatal stress is generally associated with increased peak and/or extended pituitary-adrenal response duration, though there are many variations to this general pattern [65, 66]. Basal plasma ACTH and corticosterone concentrations are elevated in female rats born to prenatally stressed dams [67, 68], and this is associated with adrenal hypotrophy [67].

A number of studies have attempted to address the effect of prenatal maternal stress in children [65, 69]. A recent study undertaken of a sample of 10-year-old children from the Avon Longitudinal Study of Parents and Children (ALSPAC) has demonstrated for the first time a significant link between prenatal anxiety, particularly in late pregnancy, and individual differences in salivary cortisol [70]. Excess amounts of corticotropin-releasing hormone (CRH) and cortisol reaching the human fetal brain during periods of chronic maternal stress could alter the personality and predispose the fetus to attention deficits and depressive illness through changes in neurotransmitter activity [65].

Few studies have compared the impact of prenatal stress on HPA activity in male and female offspring using identical prenatal protocols, and results have been variable [68, 71]. Basal ACTH, but not corticosterone, is elevated in adult female offspring born to prenatally stressed dams and pitui-
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Prenatal exposure to GSs may “program” a range of tissue-specific pathophysiologies. They suppress uterine natural killer cells, stimulate the secretion of human chorionic gonadotropin, and promote trophoblast proliferation and invasion. However, chronic administration of GSs may result in the induction of apoptosis, as well as in the inhibition of embryonic and placental growth. Their increased exposure of the fetus is associated with: IUGR, an increased risk of preterm labor and the programming of increased postnatal activity in the HPA axis.

The use of antenatal GSs (a single course of betamethasone or dexamethasone) to mature the fetal lung in pregnancies likely to deliver before 34 weeks is recommended. It reduces the incidence of respiratory distress syndrome (RDS) in the newborn, and results in neonatal mortality improvement. Premature infants exposed to antenatal GS therapy also have more circulatory stability, and are less likely to experience an intraventricular hemorrhage or necrotizing enterocolitis than unexposed preterm infants.

Rheumatologists, hematologists, dermatologists and gastroenterologists may have reason to provide systemic GSs to pregnant women. Although systemic GSs all cross the placenta to some degree, the extent to which they do so depends on the drug involved. It is highly important that to further study be carried out on the effects of glucocorticoid treatment in clinic, and how it can affect the treated children in a life perspective.

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