CLINICAL STUDY

Circulating glucocorticoid bioactivity and serum cortisol concentrations in premature infants: the influence of exogenous glucocorticoids and clinical factors

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Abstract

Objective: Glucocorticoids are widely used before preterm delivery and in preterm infants may bear serious adverse effects. Better knowledge about the circulating glucocorticoid milieu after glucocorticoid treatment could improve treatment modalities. Therefore, we investigated the influence of exogenous glucocorticoids and clinical factors on serum cortisol (F) levels and circulating glucocorticoid bioactivity (GBA) in preterm infants.

Design: Eighty-nine infants (gestational age (GA) 23.6–33.1 weeks at birth) were enrolled in a prospective cohort study in two tertiary neonatal centres.

Methods: Cord, day of birth (D0), fourth day (D4) and 36 weeks postmenstrual age serum F and GBA levels were measured.

Results: The cord GBA was 5.8-fold and D0 GBA 2.3-fold higher in the infants exposed to antenatal steroids within 12 h before birth when compared with those unexposed or exposed > 7 days before birth (95% CI 3.8–8.6; $P < 0.0001$, and 1.8–3.0; $P < 0.0001$ respectively). In the infants treated with early postnatal dexamethasone, D4 GBA was 1.7-fold (1.3–2.2; $P < 0.0005$) higher when compared with levels in the infants without this treatment. Clinical factors indicating perinatal distress, such as Apgar scores < 7 and low GA, were associated with higher cord, D0 and D4 serum F levels.

Conclusions: Both ante- and postnatally administered glucocorticoids increase circulating GBA not attributable to endogenous F. Perinatal distress and preceding glucocorticoid treatment need to be taken into account when circulating glucocorticoid milieu is evaluated in preterm infants. The GBA assay may prove to be a useful instrument in the development of new glucocorticoid treatment strategies.

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Introduction

Glucocorticoids are widely used before preterm delivery and in preterm infants (1). Antenatally administered betamethasone reduces perinatal mortality and morbidity (2), and postnatal dexamethasone (Dx) temporarily improves respiratory function and expedites extubation (3). However, early postnatal use of Dx may bear adverse effects, including those related to brain development (4–6). The most detrimental side effects may be partly due to steroid dosing, and/or to varying pharmacokinetics of glucocorticoids in preterm infants. For example, clearance of Dx is dependent on gestational age (GA), being slowest in the tiniest preterms (7). Additional complexity arises from different biological activities of the steroids in clinical use. Thus, knowledge about how the circulating glucocorticoid

milieu is influenced by exogenous glucocorticoids could improve treatment modalities.

We have recently developed a recombinant cell bioassay, based on the expression of human glucocorticoid receptor together with an appropriate reporter gene in mammalian cells (8). The assay measures glucocorticoid bioactivity (GBA) brought about by both endogenous and exogenous glucocorticoids, is capable of measuring GBA in a small amount of serum (10 µl), and thus is suitable for investigating GBA even in the most preterm infants (9).

In the current work, we analysed the influence of clinical factors and various glucocorticoid treatment modalities on cortisol (F) concentrations and GBA measured in the cord and preterm infants' serum postnatally. We anticipate that our results prove useful when optimising glucocorticoid treatment in preterm infants.

Subjects and methods

Subjects

The study population consisted of preterm infants enrolled originally in a prospective cohort study to investigate the association of adrenocortical function with the outcome. The infants were born in Kuopio or Oulu University Hospitals between October 1998 and September 2001. The entry criteria to the study were 1) prematurity (GA < 34 weeks at birth), 2) need for mechanical ventilation during the first day of life, and 3) absence of life-threatening congenital or chromosomal anomalies. The study design was approved by the Research Ethics Committees of both units. Written informed consent was obtained from the parents before the study.

Chorioamnionitis and pre-eclampsia were diagnosed based on clinical criteria by the attending obstetrician. The GA at birth was calculated from the last menstruation of the mother or by ultrasound examination of the foetus at 11 weeks postmenstrual age (PMA). Birth weight (BW) was recorded and the criterion for small gestational age (SGA) was $BW \leq -2$ s.d. according to the Finnish intrauterine growth charts (10). The mode of delivery (vaginal or caesarean section), gender and single or multiple birth were recorded. The Apgar scores were assessed at 1 min and a score below seven was considered low and an indicator of perinatal distress. The criterion for the diagnosis of bronchopulmonary dysplasia (BPD) was oxygen dependency at 36 weeks PMA.

Glucocorticoid treatments

Antenatal steroid treatment (ANS) consisted of betamethasone 12 mg twice or Dx 15 mg and 10 mg given to the mothers intramuscularly on 2 consecutive days when a preterm delivery had been anticipated. Additional ANS courses had been given at the discretion of the attending obstetrician. The time interval between the last ANS and birth was recorded in four categories: 1) < 12 h, 2) 12–72 h, 3) 3–7 days and 4) more than 7 days or no ANS. None of the mothers had received regular glucocorticoid therapy during the pregnancy.

The effect of postnatal Dx treatment on F and GBA levels was analysed at two different time points: on day 4 (D4) after 'early Dx' and at 36 weeks PMA after '36 weeks Dx'. The 'early Dx' course (median duration 2 (range 1–4) days) was used as an attempt to prevent BPD (11). The '36 weeks Dx' included 'early Dx' and later Dx treatments for infants with severe respiratory distress syndrome (RDS) or prolonged need for mechanical ventilation. It was started between days 1–35 and given for median 8 (range 1–35) days. The dose of Dx used was 0.25 mg/kg twice a day intravenously. Hydrocortisone 5 mg/kg per day was used to treat hypoglycaemia resistant to intravenous glucose administration. Adrenal insufficiency after prolonged Dx treatment was

supplemented with hydrocortisone < 1 mg/kg per day. Budesonide 500–2000 µg/day to treat BPD was administered with Spira Module 2 nebuliser (08TSM002, Spira OY, Hämeenlinna, Finland).

Laboratory measurements

Serum samples for basal F and GBA measurements were collected from mixed cord blood and venous or arterial blood on the day of birth (D0, median age 4, range 0–14 h), day 4 (D4, median age 4, range 2–7 days) and at 36 weeks (median age 36, range 33–37 weeks) PMA. Between birth and D0 sampling, none of the newborns were given Dx or hydrocortisone. The D4 and 36 weeks serum samples were collected at least 48 h after the last Dx dose. All serum samples were stored at -20°C until analysed.

Serum F was analysed by the Immulite 2000 chemiluminescent enzyme immunoassay (EIA; Diagnostic Products Corporation, Los Angeles, CA, USA). The intra- and interassay coefficients of variation (reported by the manufacturer) were below 7.5 and 9.5% respectively in the concentration range of 91–855 nmol/l. The cross-reactivity of the F antiserum was 7.5% for corticosterone, 1.6% for 11-deoxycortisol, 1.0% for cortisone and 0.2% for 17OH-progesterone and 21-deoxycortisone. There was no detectable cross-reactivity for DHEAS, progesterone or Dx. The lowest reported F value was 28 nmol/l. GBA was measured directly from 10 µl serum samples using the recombinant cell bioassay in which COS-1 cells are transfected with expression vectors encoding the human glucocorticoid receptor and the nuclear receptor coregulator androgen receptor-interacting protein 3 (ARIP3), together with an appropriate reporter gene (luciferase), as described previously (8). The lowest reported value was 15.6 nmol/l F equivalents in foetal calf serum.

Data analysis

Data were analysed using the statistical program SPSS for Windows, Release 11.5.1 (SPSS Inc., Chicago, IL, USA). Only the serum samples with both F and GBA measurements available were included in the analyses. The F levels below the detection limit (< 28 nmol/l) were recorded in the analyses as 27 nmol/l and GBA levels < 15.6 nmol/l F equivalents as 15.0. Based on previous studies (9, 12), control groups not expected to have any exogenous glucocorticoid activity were formed from the total study group separately for each sampling time. The control groups consisted of infants who had received ANS more than 7 days before birth or no ANS (cord, D0 and D4 control groups) and had not received any postnatal glucocorticoid therapy (D4 and 36 weeks control groups).

As all F and GBA values followed right-skewed distribution, logarithmically transformed values were used in linear regression models investigating the role of

different clinical variables (listed in Table 1) in explaining the variation observed in serum F and GBA. To allow for possible non-linear effects, the time between the last ANS dose and birth was divided into four categories (<12 h, 12–72 h, 3–7 days and >7 days or no ANS); the first three subsequently served as dummy variables in the regression analyses when compared with the control group. Thus, the regression coefficients represent fold-change of the dependent factor caused by a unit change in the independent factor. To make interpretation easier, the coefficients reported were back-transformed by inversed logarithmic change. In addition, Spearman's rank correlation analysis was used to investigate the strength of non-linear relationships between the two variables. In comparisons between multiple groups, Kruskal–Wallis test and between two groups, Mann–Whitney test was used (with Bonferroni correction for multiple comparisons). $P < 0.05$ was accepted to indicate statistical significance.

Results

Eighty-nine infants were enrolled in the study. The clinical characteristics of the study group are summarised in Table 1. Nine infants (10%) died and 26 (30%) contracted BPD before 36 weeks PMA. The total

Table 1 The baseline characteristics and glucocorticoid treatments of 89 preterm infants.

Gestational age (GA)	28.2 (23.6–33.1) ^a	weeks
Clinical chorioamnionitis	27 (30%)	
Pre-eclampsia	24 (27%)	
Caesarean section	65 (73%)	
Birth weight (BW)	1059 (455–2210) ^a g	
Small for gestational age (BW ≤ -2 s.d.)	21 (24%)	
Singleton	58 (65%)	
Boys	44 (49%)	
Time since last antenatal steroid (ANS)	<12 h	15 (17%)
	12–72 h	28 (31%)
	3–7 days	25 (28%)
	>7 days or no	21 (24%)
ANS courses	0	6 (7%)
	1	71 (80%)
	2	4 (4%)
	3	7 (8%)
	4	1 (1%)
Early dexamethasone treatment (Dx, before D4)	17 (19%)	
Dx before 36 weeks postmenstrual age (PMA)		
Dx treated	39 (44%)	
Number of days on Dx before 36 weeks PMA (days)	8 (1–35) ^b	
Inhaled budesonide	43 (48%)	

^aMean (range).

^bMedian (range).

number of available cord, D0 (first day), D4 (median 4th, range 2–7 days) and 36 (range 33–37) weeks serum F and GBA pairs was 42, 79, 63 and 68 respectively. The serum samples of the six infants who were treated with high dose of hydrocortisone on D4 were excluded from the D4 analyses. The median (range) serum F and GBA concentrations and the correlation coefficients between F and GBA in the infants without ongoing or recent glucocorticoid treatment are shown in Table 2. The GBA levels were 25–29% of the respective F levels and these variables displayed strong positive correlation with each other. The 36 weeks serum F was <28 nmol/l in two infants and <55 nmol/l in eight infants without any postnatal steroid treatment. They all also had unmeasurable GBA levels (<15.6 nmol/l F equivalents).

Cord and D0 serum F and GBA

The cord and D0 GBA levels were significantly higher in the infants exposed to ANS within 12 h before birth than in those exposed >7 days before birth or not at all (Fig. 1). On the other hand, the cord and D0 F levels tended to be lower in the infants with recent exposure to ANS than in those with remote or no exposure ($P = 0.12$ and $P = 0.20$ respectively). Cord F level was 1.7-fold (95% CI 1.1–2.6; $P < 0.04$) and D0 serum F level 2.6-fold (95% CI 1.7–4.0; $P < 0.0001$) higher in infants with Apgar scores <7 compared with those with Apgar ≥ 7 , when the model was adjusted for timing of ANS, number of ANS courses, mode of delivery and GA. These factors explained approximately 37 and 26% of the variation observed in cord and D0 F levels respectively. The timing and number of ANS courses were the only significant determinants of the cord GBA levels (Table 3A), whereas the timing of ANS and serum F levels were significant determinants of D0 GBA levels (Table 3B).

D4 serum F and GBA

The D4 GBA levels were higher in the infants treated with the 'early Dx' treatment than in controls (Fig. 2),

Table 2 The median (range) serum cortisol (F) concentrations, glucocorticoid bioactivity (GBA) levels and Spearman correlation coefficients (r) between the respective serum F and GBA values in the infants without ongoing or recent glucocorticoid treatment.

Sampling time	<i>n</i>	F (nmol/l)	GBA (nmol/l cortisol equivalents)	<i>r</i>
Cord	12	86 (51–147)	23 (<15.6–44)	0.625*
D0 ^a	18	453 (50–2307)	127 (<15.6–315)	0.979†
D4 ^a	12	175 (45–340)	50 (16–96)	0.958†
36 weeks ^a	27	142 (<28–394)	35 (<15.6–135)	0.876†

* $P < 0.03$, † $P < 0.0001$.

^aD0 median age 4, range 0–14 h; D4 median age 4, range 2–7 days; 36 weeks median age 36, range 33–37 weeks; postmenstrual age.

Figure 1 The cord and first day (D0) serum glucocorticoid bioactivity (GBA) and cortisol levels (medians, 25th and 75th percentiles) in premature infants grouped according to the time since the last antenatal steroid (ANS) dose before birth (<12 h, 12–72 h, 3–7 days (open columns) and >7 days or no ANS as controls (filled columns)). The median cord and D0 serum GBA were significantly higher in the infants treated with ANS <12 h before birth compared with the controls ($^{\dagger}P<0.0001$; $^*P<0.05$; Kruskal–Wallis test followed by Mann–Whitney test with Bonferroni correction for multiple comparisons).

Table 3 Factors contributing to the cord (A), first day (D0; B) and fourth day (D4; C) circulating glucocorticoid bioactivity (GBA) in premature infants in multiple linear regression models.

Time since the last ANS dose before birth						
A cord (n=42)	0–12 h	12–72 h	3–7 days	Number of ANS courses	Apgar <7	Log of cord serum cortisol (nmol/l)
B coefficient (95% CI)	5.8 (3.8–8.6)	1.4 (0.94–2.0)	1.1 (0.69–1.6)	0.78 (0.62–1.0)	1.2 (0.87–1.6)	1.5 (0.85–2.5)
P value	<0.0001	0.10	0.79	0.03	0.27	0.16
R ² =0.78						
Time since the last ANS dose before birth ^a						
B D0 (n=79)	0–12 h	12–72 h	3–7 days	Number of ANS courses	Apgar <7	Log of D0 serum cortisol (nmol/l)
B coefficient (95% CI)	2.3 (1.8–3.0)	1.3 (1.0–1.6)	0.93 (0.73–1.2)	0.89 (0.80–1.0)	1.0 (0.82–1.2)	3.6 (2.9–4.4)
P value	<0.0001	0.03	0.52	0.05	0.99	<0.0001
R ² =0.78						
Time since the last ANS dose before birth ^a						
C D4 (n=63)	0–12 h	12–72 h	3–7 days		'Early Dx' treatment ^b	Log of D4 serum cortisol (nmol/l)
B coefficient (95% CI)	1.3 (0.96–1.7)	1.1 (0.81–1.4)	1.1 (0.81–1.4)		1.7 (1.3–2.2)	4.5 (3.2–6.2)
P value	0.09	0.59	0.66		<0.0005	<0.0001
R ² =0.74						

^aThe reference group was the infants with no antenatal steroid treatment (ANS) or the last ANS dose at least 7 days before birth. Due to the logarithmic transformation of the GBA values, the regression coefficients are back-transformed by inversed logarithmic change and represent B-times higher GBA levels produced by a unit change in the factor. All the models were adjusted for the gestational age at birth, and cord and D0 models for mode of delivery. CI, confidence interval.

^b'Early Dx' dexamethasone treatment median 2 (range 1–4) days at least 48 h before D4 sampling.

Figure 2 The fourth day (D4) serum glucocorticoid bioactivity (GBA) and cortisol levels (medians, 25th and 75th percentiles) in premature infants treated with an early short-term dexamethasone course (Dx, open columns) compared with those without this treatment (No, filled columns). The median D4 serum GBA was significantly higher in the Dx treated than in the non-treated infants (* $P < 0.005$, Mann–Whitney test).

but no such difference was found in D4 serum F levels. In the multiple linear regression analysis, 1 week reduction in GA was associated with 1.1-fold (95% CI 1.0–1.2, $P < 0.05$) D4 F levels, when the model was adjusted for the timing of ANS and the ‘early Dx’ treatment ($R^2 = 0.29$). The ‘early Dx’ treatment and the D4 serum F levels were the only significant contributors to the D4 GBA levels (Table 3C).

Thirty-six weeks serum F and GBA

The median 36 weeks serum F was significantly lower in the Dx-treated infants than in the non-treated infants (43 vs 107 nmol/l, $P < 0.02$, $n = 68$) and the median 36 weeks serum GBA had a similar trend (19 vs 22 nmol/l

cortisol equivalents, $P = 0.12$). In addition, the duration of the ‘36 weeks Dx’ treatment correlated negatively with the 36 weeks serum F levels ($r = -0.301$, $P < 0.02$) and tended to correlate with the 36 weeks serum GBA levels ($r = -0.211$, $P = 0.08$). Similarly, the inhaled budesonide treatment preceding 36 weeks correlated negatively with the 36 weeks serum F and GBA levels (data not shown). Because numerous study subjects had received both inhaled budesonide and ‘36 weeks Dx’ therapy, we were unable to distinguish the effects of these two steroids on serum GBA and F levels.

Discussion

To our knowledge, this is the first study to measure circulating GBA levels in preterm infants after birth. In preterm infants not recently exposed to exogenous glucocorticoids, we found a strong correlation between serum F and GBA levels at all time points investigated postnatally. This is in concordance with two previous studies, in which serum F and GBA levels were measured in newborns and children (8, 9). After some reports had linked the use of early postnatal Dx in preterm infants to suboptimal long-term brain development (4–6), new strategies in glucocorticoid use have been developed. For example, the recently introduced early hydrocortisone prophylaxis possibly increases survival without BPD (13, 14). However, an increased risk of gastrointestinal perforations associated with high endogenous F levels was noted (14). The bioassay for serum GBA, employed in the current work, provides a novel means to evaluate the effects of glucocorticoid treatment on circulating glucocorticoid milieu, and may thus pave the way for safer therapy of infants born prematurely.

Preterm infants treated with ANS within the 12 h before birth displayed the highest serum GBA levels. This is in agreement with previous studies (9, 12) and underlines the fact that betamethasone and Dx are poor substrates for the 11β -hydroxysteroid dehydrogenase type 2 enzyme, the metabolic barrier protecting the foetus against high maternal F concentrations (15). The lack of significant differences in the cord and D0 serum F levels between the different ANS treatment groups is at least partly explained by the small number of infants without ANS treatment making the type two statistical error possible. However, the tendency to lower F levels in the infants treated with ANS within 72 h before birth is in concordance with previous studies (9, 12, 16–18). The association of decreased cord and D0 serum GBA with the increasing number of ANS courses is probably mediated by the suppression of the respective F levels after multiple ANS courses administered several days before delivery (17, 19).

Treatment of a preterm infant with Dx for a week or longer is associated with suppression of the hypothalamo–pituitary–adrenal (HPA) axis (20–24), and therefore shorter Dx courses have been introduced to

treat severe RDS and to wean the infant from mechanical ventilation (11, 17, 25–28). In some studies, even 2–5 days of Dx treatment has been shown to suppress F levels (17, 27), although this is not a constant finding in all studies (28). In our work, GBA levels were still high 48 h after ‘early Dx’ treatment, a finding probably reflecting the long half-time of Dx in the most premature infants (7). In contrast, following a longer Dx course both F and GBA levels at 36 weeks PMA were suppressed, probably reflecting central suppression of the HPA axis. The lack of significant correlation between serum GBA levels at 36 weeks PMA and the duration of such longer Dx courses might be explained by the influence of the ongoing inhaled budesonide treatment (8).

Low Apgar scores and GA are associated with increased cord, D0 and D4 F and GBA levels. This suggests that most preterm infants have functional HPA axis with appropriate capacity to secrete F in response to distress, as proposed also in previous studies (29–31). However, there may be preterm newborns who have significant adrenocortical insufficiency in relation to their clinical condition during the first days of life (32), and who might benefit from glucocorticoid treatment (13, 14). On the other hand, and quite surprisingly, we found several healthy infants whose F and GBA levels at 36 weeks PMA were very low, even without any postnatal glucocorticoid treatment. Thus, one of the future challenges related to optimising glucocorticoid treatment is to find the appropriate serum F range in premature infants in different degrees of distress.

In conclusion, both ante- and postnatally administered glucocorticoids increase circulating GBA not attributable to endogenous F. The preceding glucocorticoid treatment and the increase in endogenous F caused by birth-related distress need to be taken into account when evaluating circulating glucocorticoid milieu in preterm infants. The GBA assay may prove to be a useful instrument in the development of new glucocorticoid treatment strategies.

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