Glucocorticoid Levels in Maternal and Cord Serum after Prenatal Betamethasone Therapy to Prevent Respiratory Distress Syndrome

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ABSTRACT Serum glucocorticoid levels were determined in 20 mothers and 43 premature infants who received prenatal betamethasone therapy for prevention of respiratory distress syndrome (RDS). Maternal betamethasone peaked at 75 μg cortisol equivalents per 100 ml 1 h after injection of 12 mg steroid and declined to half by 6 h. Betamethasone in cord blood was 14.3 μg cortisol equivalents per 100 ml at 1 h, decreased to a level of 4.7 at 20 h, and was not detected 2 days after a second dose at 24 h. After the second dose, the mean level of cortisol in cord blood was 5.9 μg per 100 ml compared with 13.05 μg per 100 ml ($P < 0.001$) in untreated premature infants. The unbound glucocorticoid activity in treated infants delivered 1–10 h after the second dose (mean, 8.4 μg per 100 ml) is similar to the unbound cortisol level after birth in untreated premature infants who develop RDS.

These findings indicate that (*a*) serum glucocorticoid levels in the physiologic stress range can induce lung maturation in the human and (*b*) antenatal treatment with this dose of betamethasone does not expose the human fetus to potentially harmful pharmacologic levels of steroid.

INTRODUCTION

Administration of glucocorticoids to fetal animals of several species accelerates lung development and causes precocious appearance of alveolar surfactant (3–5). Since a deficiency of surfactant is the major cause of respiratory distress syndrome (RDS)[1] in premature human infants, these findings suggested that glucocorticoids might benefit the human infant at risk for this disease. Recently, Liggins and Howie (6, 7) reported a significantly lower incidence of RDS in premature infants whose mothers were treated with betamethasone for at least 24 h before delivery. In a similar study, Fargier et al. (8) also noted a beneficial effect of prenatal betamethasone therapy. These studies have indicated the effectiveness of prenatal glucocorticoid treatment for prevention of RDS, and it seems likely that such therapy will come into general use in the future.

At present, however, little is known regarding the level of glucocorticoid required to induce lung maturation in human infants. Also, there is little information concerning the safety of prenatal corticosteroid administration. It does appear that the lung is a glucocorticoid target tissue since both animal (9, 10) and human (11) fetal lung contain specific receptors for glucocorticoids. These binding proteins are saturated by physiologic levels of corticosteroids, suggesting that the desired maturational effects should be accomplished without the use of pharmacologic doses of steroid. This is an important consideration since it is known that large doses of corticosteroids inhibit growth in many organs of fetal and neonatal animals (12–18).

In the present study, we have determined serum levels of glucocorticoid activity in the human mother and fetus at various times after prenatal treatment with betamethasone to prevent RDS. The findings are discussed in relationship to the safety of this therapy and in re-

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[1] *Abbreviations used in this paper:* CBG, corticosteroid-binding globulin (transcortin); PROM, prolonged rupture of membranes; RDS, respiratory distress syndrome.

gard to the mechanism of action of glucocorticoids in lung development.

METHODS

Treatment of patients. Women admitted in premature labor to Mount Zion Hospital, San Francisco, and to the University of California, San Francisco, were treated according to the protocol of Liggins and Howie (6) with an i.m. administration of a mixture of 6 mg betamethasone phosphate and 6 mg betamethasone acetate (Celestone Soluspan, Schering Corporation, Kenilworth, N. J.). An informed consent was obtained before treatment. Labor was delayed in most cases by intravenous alcohol and, whenever possible, a second dose of betamethasone was administered 24 h after the first. Samples of maternal venous blood were obtained before and at various times after treatment, and mixed cord bloods were collected when delivery occurred within 4 days after the first dose. Additional cord blood specimens were obtained from premature infants delivered at the National Women's Hospital, Auckland, New Zealand, to women who had received either the same betamethasone therapy or a placebo (6).[2] Serum was separated by centrifugation within 48 h from both maternal and cord samples and stored at − 70°C.

Infant gestational age was assessed by obstetrical data and by physical and neurologic development. Statistical significance was evaluated by Student's unpaired *t* test and by the method of least squares.

Steroid assays. The glucocorticoid radioreceptor assay was performed as previously described (19). In brief, duplicate aliquots of 0.05–0.1 ml of sera were extracted twice with 10 vol of absolute ethanol. The extract was brought to dryness under a gentle stream of air, and the residue was dissolved in 0.1 ml of buffer. Before ethanol extraction cord serum samples were washed twice with 10 vol of petroleum ether (20), which removes 90–93% of the progesterone. The extracted steroids were incubated at 2°C for 18 h with [^{3}H]dexamethasone and cytosol (which contains glucocorticoid receptor activity) of cultured hepatoma cells. Unbound [^{3}H]dexamethasone and receptor-bound steroid were separated by the charcoal absorption technique. The amount of glucocorticoid activity in the sample was determined by comparison with plasma standards containing known amounts of cortisol.

In order to determine the amount of glucocorticoid activity that was due to betamethasone, serum corticoids were also assayed in the ethanol extracts of each sample by the corticosteroid-binding globulin (CBG)-isotope method of Murphy (20) and the charcoal absorption technique (19). This assay does not detect betamethasone. Appropriate control sera (without synthetic corticosteroids) were included in each experiment to establish the correlation between results in the two assays. The level of betamethasone was then determined by the amount of excess activity found by radioreceptor assay as compared with the CBG-isotope assay. Serum concentrations are expressed as cortisol equivalents and reflect the relative affinity of betamethasone for glucocorticoid receptors. Values may be converted to micrograms betamethasone per 100 ml by dividing by 7.1 (19). The assay under these conditions is sensitive to a level of 2 μg cortisol equivalents per 100 ml.

The percentage of serum cortisol bound to transcortin was determined in undiluted sera by a modification of the absorption method of Trapp and West (21) with a tracer amount of [^{3}H]cortisol and absorption by activated charcoal as previously described (19). This method measures binding to transcortin (but not to albumin) and provides results similar to those found by ultrafiltration and equilibrium dialysis at 37°C.[3] Transcortin binding was assessed in pooled cord serum samples from premature infants after addition of various amounts of unlabeled cortisol. Transcortin capacity in these and other samples was calculated from the amount of binding at a cortisol level of 50 μg per 100 ml (21). The amount of unbound cortisol and cortisol bound to albumin was estimated (22) from these data by using published values for cord blood albumin levels in premature infants with and without RDS (23).

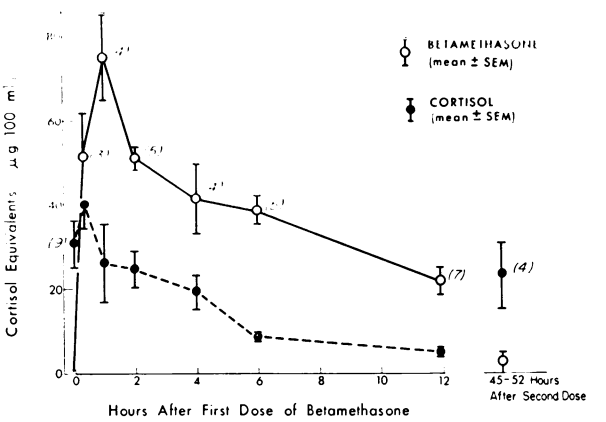

FIGURE 1 Betamethasone and cortisol levels in maternal serum. The number of samples at each time point is shown in parentheses.

RESULTS

Maternal steroid levels. Determinations of betamethasone and cortisol levels were carried out on 37 samples of blood from 20 women 0–12 h after the first dose of steroid. Four additional samples were obtained 45–52 h after a second dose (Fig. 1). The level of betamethasone is maximal (75 μg cortisol equivalents per 100 ml) at 1 h and then declines to half of this value at about 6 h; little activity remained in the maternal circulation 2 days after the second injection. Cortisol levels begin to decrease within 2 h of the administration of exogenous corticosteroid and reach a level of 5 μg per 100 mg ml at 12 h. During the 2nd day of treatment, cortisol was below 5 μg per 100 ml in four of five patients (data not shown). 2 days after the second injection, the mean level of cortisol in five women at delivery was within the normal range.

Betamethasone levels in cord blood. Steroid levels were measured in samples of cord blood from 43 premature infants exposed to betamethasone in utero. Cord bloods from another group of 22 infants who were delivered at comparable times after treatment with a

[2] We thank G. C. Liggins and R. N. Howie for providing these samples.

[3] P. Ballard. Data to be published.

placebo (6) served as controls in the betamethasone assay and for comparison of cortisol levels. All infants in both groups were delivered vaginally after spontaneous labor except for one treated infant, who was delivered by cesarean section. The mean gestational age and birth weight, sex distribution, and incidence of prolonged rupture of membranes (PROM) were similar in the two groups (Table I).

Individual cord blood betamethasone results are shown on a time scale in Fig. 2. Steroid is detected as early as 1 h after the first dose. By regression analysis the mean level of betamethasone is 14.3 μg cortisol equivalents per 100 ml at 1 h and declines with a slope of -0.50 ± 0.16 (SD) ($P < 0.01$) to 4.7 μg per 100 ml at 20 h. Somewhat higher values were found between 1 and 10 h after the second dose; the mean level for these samples was 15.0 μg cortisol equivalents per 100 ml compared with 12.0 μg per 100 ml for the same time period after the first injection. It is of interest that the three samples with the highest levels of betamethasone (29.6, 32.8, and 23.6 μg cortisol equivalents per 100 ml) were from the three infants born to women with moderate or severe antepartum hemorrhage. Betamethasone was not detected (< 2 μg per 100 ml) in cord blood obtained from five infants born between 62 and 72 h after the first dose.

Cortisol levels in cord blood. Cortisol levels for the treated and control infants are presented in Fig. 3. Values are significantly suppressed in most treated infants born after betamethasone treatment. Mean values for treated infants were 7.45 and 5.9 μg per 100 ml after the first and second dose of betamethasone, respectively. Both values are significantly lower ($P <$ 0.001) than the means for control infants (14.65 and 13.05 μg per 100 ml). Cortisol remains depressed (mean, 4.95 μg per 100 ml) in the five samples obtained during the 3rd day after treatment.

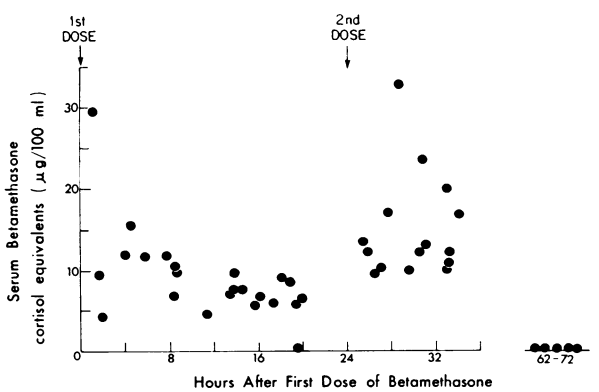

FIGURE 2 Betamethasone levels in cord bloods of premature infants. Individual data for 43 infants are shown.

TABLE I
*Characteristics of Premature Infant Groups Treated with Betamethasone or Placebo**

	Betamethasone	Placebo
Number	43	22
Gestational age,‡ wk	32.7±0.40 (27–36.5)	32.7±0.60 (26.5–36)
Birth wt,‡ g	1,840±82 (500–2,680)	1,987±115 (800–2,880)
Male:female	26:17	11:11
PROM (>24 h)	12/43 (28%)	4/22 (18%)

* Includes data provided by G. C. Liggins and R. N. Howie.
‡ Values are mean±SEM and range.

Comparison of maternal and fetal steroid levels. A comparison of the data in Figs. 1 and 2 suggests that fetal levels of betamethasone are lower than maternal levels during at least the first 12 h of treatment. Higher maternal levels were found in each of six matched maternal and cord blood samples which were obtained at various intervals (1–61 h) after the first dose of betamethasone (Table II). The mean gradient was 3:1 with a range from 1.6:1 to 4.1:1.

Maternal:fetal gradients also were present for cortisol (mean, 5.8:1), and in most cases these were somewhat greater than for betamethasone (Table II). In one instance (case 7), the fetal sample was collected from the umbilical artery 2 h after birth, when the infant had already developed mild RDS. Cortisol was elevated in this infant (22.4 μg per 100 ml) compared with cord values of other infants delivered after a similar treatment interval (cf. Fig. 3). In four other premature newborn infants with mild or moderate RDS delivered 52, 68, 82, and 95 h after the first dose of betamethasone, cortisol levels at 13–24 h of life were 28.9, 11.4, 19.0, and 29.9 μg per 100 ml.

Unbound glucocorticoid levels in cord blood. The data presented thus far have described the total amount of plasma glucocorticoid activity. Since it is generally felt that unbound glucocorticoid and perhaps albumin-bound steroid are the physiologically active fractions, we determined the amounts of transcortin-bound and non-transcortin-bound steroid in cord bloods. In agreement with earlier reports (24) we found that the binding capacity is lower in cord than in adult blood. The transcortin capacities of pooled control and treated cord bloods from the premature infants were 8.3 and 9.6 μg cortisol per 100 ml, respectively, compared with mean values of 10.8±1.1 (SEM) μg per 100 ml ($n = 7$) in term infant cord bloods and 20.3±1.7 μg per 100 ml ($n = 10$) in adult samples. At cortisol levels of 5.9, 10.5, 16.2, and 22.1 μg per 100 ml in premature infant cord serum, the percentage of steroid not bound to trans-

TABLE II

Betamethasone and Cortisol Levels in Matched Maternal and Cord Bloods at Delivery

Case no.	First treatment delivery interval	Gestational age	Birth wt	Betamethasone		Cortisol	
				Maternal	Cord	Maternal	Cord
	h	*wk*	*g*	*μg cortisol eq/100 ml*			
1	1.0	31	1,600	74.4	29.5	51.0	6.6
2	4.5	34	2,345	49.6	15.4	76.5	12.0
3	15.5	32	1,830	8.9	2.8	15.6	2.9
4 Twin A	33	27	800	31.9	10.3	29.4	5.1
Twin B	33	27	500	31.9	19.9	29.4	8.4
5	61	34	2,180	8.3	2.0	35.0	9.8
6	68.5	30	1,247	<2	<2	30.4	3.7
7	72	29	1,430	<2	<2*	20.9	22.4*

* Sample obtained 2 h after birth; infant had mild RDS.

cortin was found to be 49, 53, 59, and 64%, respectively. The ratio of albumin-bound and unbound cortisol within this fraction has been shown previously to depend only on the albumin concentration (22). Betamethasone is not bound to transcortin (19, 25), and from the studies of Peets et al. (25) we estimate that 47% is unbound in cord blood of the premature infant.

Using these binding data, we have compared the levels of total, albumin-bound, and unbound glucocorticoid activity in betamethasone-treated infants with the levels in normal and stressed premature infants (Table III). In our study, the mean cortisol level in cord blood of 14 untreated premature infants born without PROM or other maternal or fetal complications was 10.5 μg per 100 ml, resulting in 2.8 μg albumin-bound and 2.8 μg unbound cortisol per 100 ml. In other studies, mean total cortisol levels of about 16 and 22 μg per 100 ml have been reported for infants who are born after PROM or who develop RDS shortly after birth, respectively (26–28). We also have found similarly elevated cortisol values in a limited number of infants under both conditions (data not presented). The unbound cortisol levels in these two situations are 4.6 and 7.8 μg per 100 ml, which are in the order of 1½ and 3 times that of normal premature infants. In treated infants, both cortisol and betamethasone contribute to the plasma glucocorticoid activity. We calculate that these infants have peak unbound and albumin-bound glucocorticoid levels of 9.4 and 8.4 μg cortisol equivalents per 100 ml, respectively. Betamethasone treatment at this dose, therefore, results in an unbound glucocorticoid level similar to that achieved by endogenous cortisol production in the premature infant with RDS.

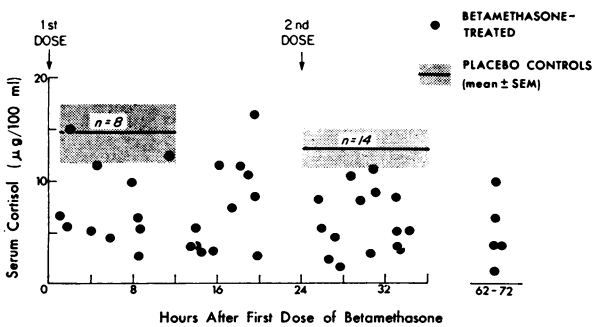

FIGURE 3 Cortisol levels in cord bloods of betamethasone-treated and control premature infants. Individual cortisol values of treated infants are compared with mean values for control infants.

DISCUSSION

We have determined serum levels of betamethasone, using a radioreceptor assay for glucocorticoids. This

TABLE III

Total, Albumin-Bound, and Unbound Plasma Glucocorticoid Activity in the Premature Infant

Clinical status	Plasma glucocorticoid activity		
	Total	Albumin-bound	Unbound
	μg cortisol eq/100 ml		
Normal premature infant	10.5	2.8	2.8
PROM (>16 h)	16.2*	4.6	4.6
RDS (4 h of age)	22.1‡	6.4	7.8
Betamethasone-treated			
Cortisol	5.9§ } 20.8	1.4 } 9.3	1.4 } 8.4
Betamethasone	14.9§ }	7.9 }	7.0 }

* Mean cord plasma cortisol value in 10 premature infants studied by Bauer et al. (26).

‡ Mean plasma cortisol value in 24 premature infants studied after birth by Baden et al. (27).

§ Mean values 1–10 h after second dose of betamethasone (see Figs. 2 and 3).

method measures steroids with glucocorticoid activity in proportion to both their concentration and their affinity for the cytoplasmic receptor. It is felt that the latter property reflects the intrinsic biologic potency of corticosteroids. In this regard we have reported previously that the relative affinities of many natural and synthetic corticosteroids for receptors of hepatoma cells (used in the assay) and human fetal lung are similar (19). For this reason, we have expressed betamethasone concentrations in terms of cortisol equivalents and have directly compared serum levels of administered and endogenous glucocorticoids.

In both large studies reported to date (6–8), prenatal betamethasone therapy has significantly reduced the incidence of RDS in premature infants. We have determined the serum levels of steroid at various times after injection of the same dose of betamethasone acetate and betamethasone phosphate. In maternal serum peak levels of betamethasone occur within 1 h and decline with a half-time of about 6 h. In cord blood, betamethasone is detected within 1 h of treatment and steroid levels decrease with a half-time of about 14 h. At all times during treatment, there is less betamethasone in fetal than maternal serum with a mean gradient of 3:1 in matched comparisons. A larger gradient was found for endogenous cortisol (mean, 5.8:1). It is reported that the maternal:fetal gradient for prednisolone is about 10:1 after infusion of either prednisone or prednisolone (29). Thus, betamethasone seems preferable to cortisol, prednisone, or prednisolone for prenatal therapy since relatively lower doses need be administered to the mother.

It would appear that the mean level of betamethasone in the cord bloods of our series decreases to about 3 μg cortisol equivalents per 100 ml before the second dose at 24 h. This produces an unbound glucocorticoid activity of 3.3 μg per 100 ml, which is similar to that found in untreated infants, suggesting that the level of betamethasone in some infants may be less than optimal for several hours before the second dose. For this reason, it would seem appropriate in subsequent clinical trials with this preparation of steroid to repeat a 12-mg dose more frequently (e.g., 12 mg at 0 and 18 h). It should also be noted that the plasma glucocorticoid activity in treated infants falls below control levels during the 3rd day. It is possible that a longer exposure to elevated steroid levels (e.g., a third dose 24 h after the second) would increase the effectiveness of therapy.

As reported previously (6), therapy with this dose of synthetic corticosteroid rapidly suppresses maternal cortisol levels. Cortisol in cord blood is also decreased soon after treatment; values were lower after the second injection at a time when betamethasone levels were highest and maternal cortisol quite low. These lower values in cord blood probably reflect decreased cortisol production in the fetus as well as in the mother. However, in five treated infants with RDS, we found elevated cortisol levels after birth, suggesting that the normal pituitary-adrenal response to stress is not lost with treatment.

Another concern regarding the use of prenatal corticosteroids in human infants is the possibility of adverse effects on growth. Administration of these steroids to fetal and neonatal animals can seriously impair organ and somatic growth and neurologic function (12–18). Only some of these effects are partially or fully reversed by compensatory catch-up growth after withdrawal of steroid (12, 13, 16, 17, 30). However, the doses of hormone per kilogram body weight used in the animal studies are 15–300 times that which the human fetus receives with prenatal betamethasone therapy. This is a critical consideration since the extent of the effects in animals is dose related (12, 13, 18).

Harmful effects of high doses of corticosteroids administered to the human infant in the neonatal period are also reported (for review see reference 31) and include impaired growth and increased incidence of central nervous system abnormalities. We estimate that the glucocorticoid levels in those infants were at least 10 times greater than the maximal levels which we found in the premature infants of this study. Thus, it appears that the dose of prenatal betamethasone used in this study should not expose the premature infant to potentially harmful pharmacologic levels of plasma glucocorticoid activity. The apparent safety of betamethasone at this dose is supported by preliminary observations on the neonatal clinical course of premature infants treated in utero (6, 32).

In addition, we find only a threefold maximal increase in the unbound glucocorticoid level in premature infants exposed to prenatal betamethasone therapy. This level can be compared to the 1½- and 3-fold increases found in premature infants who are delivered after PROM or who develop RDS after birth, respectively. Thus, such treatment exposes the infant to corticoid doses no higher, and for no longer, than those which occur by endogenous production in the infant with RDS. These comparisons suggest that antenatal betamethasone treatment at this dose closely approximates a physiologic stress response. It seems unlikely, though unproven, that this level of corticoid is hazardous to the premature infant.

Our findings also indicate that prevention of RDS occurs with serum glucocorticoid levels in the physiologic stress range, suggesting that such levels are sufficient to induce lung maturation in the human fetus. This is consistent with a direct glucocorticoid effect in lung mediated by the receptor system since the levels

of serum glucocorticoid activity after treatment would be sufficient to fill all receptor sites and thereby evoke an optimal response. The elevated corticoid levels associated with PROM and RDS suggest that endogenous cortisol may act by the same mechanism to accelerate pulmonary development in these situations. It is also possible that during normal gestation the appearance of alveolar surfactant is triggered by an increase in endogenous cortisol levels. In this regard, Murphy (33) has reported that higher levels of cortisol in cord blood are associated with less RDS, and Fencl and Tulchinsky (34) have noted an increase in amniotic fluid cortisol after 34 wk gestation in normal pregnancies. Thus, the level of endogenous corticosteroid may be an important regulatory factor in both the pathogenesis and prevention of this disease. In this case, administration of prenatal glucocorticoids in appropriate circumstances may accelerate developmental events in the lung through normal physiologic mechanisms.

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