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The Effect of Betamethasone on Neonatal Neutrophil Chemotaxis

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ABSTRACT. Antenatal maternal glucocorticoid administration has been widely used to accelerate fetal lung maturation. Glucocorticoids have also been used postnatally in selected neonates as antiinflammatory agents. Numerous studies have shown that glucocorticoids inhibit multiple components of the immune system including neutrophil (PMN) function in children and adults. Since PMNs are of critical importance in host defense against bacterial infection, impaired PMN function in newborn infants is thought to be an important cause of their increased morbidity and mortality from bacterial infection. Further compromise of neonatal PMN function by exogenous factors such as glucocorticoids may therefore be of significant clinical importance. A micropore filter chemotactic assay was used to determine the *in vitro* effect of betamethasone on the random migration and directed migration (chemotaxis) of PMNs from 18 neonates. The addition of a concentration of betamethasone (0.01 μg/ml) similar to that found in cord blood following a standard dose administered to the mother resulted in a significant ($p < 0.01$) inhibition in mean neonatal PMN random migration (−15.0 ± 0.8%) and chemotaxis (−23.5 ± 3.0%). A similar inhibition was not found when PMNs from 14 adults were exposed to the same concentrations of betamethasone. Betamethasone administration to pregnant women or their newborn infants may further impair PMN motility and lead to an increased morbidity and mortality from bacterial infection in neonates. (*Pediatr Res* 22: 150–152, 1987)

Abbreviations

PMN, polymorphonuclear leukocyte
NBT, nitroblue tetrazolium
RDS, respiratory distress syndrome

Sepsis is a leading cause of mortality in the intensive care nursery, in part because of immaturity of neonatal host defenses (1). PMNs are the major cellular elements which defend against bacterial invasion. PMNs from term infants demonstrate functional deficiencies in adherence (2, 3), chemotaxis (3–5), oxidative metabolism (6–8) and intracellular killing (9, 10). Further impairment in PMN function is thought to contribute to an even greater morbidity and mortality from infection in stressed or preterm infants.[6, 9, 11]

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Antepartum maternal administration of corticosteroids (betamethasone) has been used to accelerate fetal lung maturity in selected cases of anticipated premature delivery (12, 13). Corticosteroids have been shown to inhibit several aspects of PMN function in children and adults but there are limited data in the fetus and newborn (14, 15). The objective of this study was to determine whether neonatal PMN motility would be impaired after incubation of PMNs in betamethasone (Celestone, Schering Corporation, Kenilworth, NJ) at concentrations similar to those found in cord blood following a standard 12-mg intramuscular dose administered to the mother.

MATERIALS AND METHODS

Collection and preparation of PMNs. Cord blood was obtained at the time of elective, uncomplicated cesarean section from healthy term infants whose mothers were on no medications other than vitamins and who received only local anesthesia during delivery. Blood was also obtained from healthy adult controls in accordance with hospital institutional review guidelines. Blood was anticoagulated with preservative-free sodium heparin (50 U heparin/ml blood) and mixed with 6% hetastarch in 0.9% sodium chloride (Hespan, American Hospital Supply, Irvine, CA; 0.2 ml Hespan/ml blood) to facilitate the separation of PMNs from other blood elements. The leukocyte containing plasma fraction was centrifuged for 5 min at 500 × *g* and 4° C. The resulting pellet was resuspended in Medium 199 (Difco, Detroit, MI) and PMNs were counted using a hemocytometer. The cells were then diluted with Medium 199 to a standard concentration of 5×10^6/ml.

Preparation of betamethasone. Celestone Soluspan (Schering Corporation, Kenilworth, NJ), a suspension of betamethasone acetate in an equal concentration of betamethasone sodium phosphate, was centrifuged for 5 min at 2000 × *g*. The supernatant containing 3 mg betamethasone sodium phosphate/ml was recentrifuged for 5 min at 2000 × *g* in order to eliminate insoluble betamethasone acetate particles which could act as a stimulus to neutrophil oxidative metabolism. The absence of insoluble particles which would stimulate oxidative metabolism was confirmed using an NBT assay. No differences in NBT test results were noted between PMNs from neonates or adults incubated in test tubes containing either betamethasone or a buffer solution. Serial dilutions of the betamethasone sodium phosphate were then made in buffered Medium 199 ranging from 0.005 to 10 μg/ml. All steroid-containing mixtures were shielded from light with aluminum foil to prevent diminution of potency.

Preparation of the chemoattractant. Fresh blood was obtained daily in a sterile tube without anticoagulant from a healthy adult human volunteer. After clotting had occurred, the blood was centrifuged for 10 min at 800 × *g*. The serum fraction was mixed with 0.5 mg Zymosan A (Sigma Chemical Corp., St. Louis, MO)

per 0.1 ml serum and activated by incubation for 30 min in a shaking water bath at 37° C.

Measurement of cell migration. A modified micropore filter assay was used to measure random migration and chemotaxis (16). Nitrocellulose filters with a 5-μ pore size and 13-mm diameter (Schleicher and Scheull, Inc., Keene, NH) were used. The lower compartment of the Boyden chamber contained either Medium 199 for determination of random migration or 3% zymosan-activated human serum for determination of chemotaxis. PMNs (5×10^5) suspended in Medium 199 were added to various concentrations of betamethasone and incubated for 30 min at 37° C and placed in the upper compartment of the Boyden chamber. All chambers were incubated for 90 min in a 37° C incubator containing humidified 5% CO_2-air mixture. Each assay was performed in triplicate for a given steroid concentration. After incubation, the filters were stained, mounted on slides, and examined under the microscope. The "leading front" of PMN migration was determined as the furthest distance to which at least two PMNs had migrated through the filter.

ANALYSIS OF RESULTS

The percent inhibition (%I) of PMN chemotaxis caused by *in vitro* incubation of PMNs in betamethasone was calculated from:

$$\%I = 1 - \frac{R_B}{R_0} \times 100$$

where the responses (R) are: R_B, in the presence of betamethasone and R_0, in the absence of betamethasone. The data conformed to a rectangular hyperbola of the form:

$$\%I = \frac{\%I_{max} \times [\text{betamethasone}]}{ID_{50} + [\text{betamethasone}]}$$

where $\%I_{max}$ is the maximum reversal of chemoattractant effect expected when the betamethasone concentration is extrapolated to infinite concentration and ID_{50} is the concentration of betamethasone that will produce $0.5 \times \%I_{max}$. The data were analyzed by nonlinear regression using the NLIN SAS version 5 procedure.

For other comparisons, analysis of variance (Dunnett's test) was used to determine significance with a value of $p < 0.05$ considered significant.

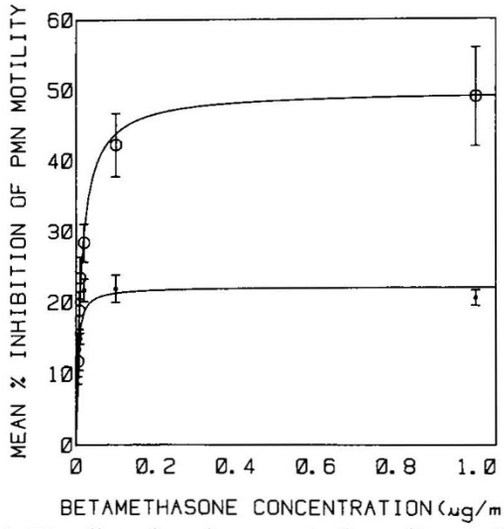

Fig. 1. The effect of varying concentrations of betamethasone on neonatal PMN random migration and chemotaxis. PMNs from 18 neonates were incubated for 30 min in varying concentrations of betamethasone and PMN random migration and chemotaxis were determined. The mean percent inhibition values ± 1 SE of PMN random migration (*closed symbol*) and chemotaxis (*open symbol*) are shown for each betamethasone concentration tested. The mean buffer control values are 52.1 ± 13.7 μm for random migration and 92.4 ± 16.8 μm for chemotaxis. The configuration of the inhibition curves most closely approximate rectangular hyperbola saturation curves of the form:

$$\%I = \frac{\%I_{max} \times [\text{betamethasone}]}{ID_{50} + [\text{betamethasone}]}$$

where $\%I_{max}$ is the maximum reversal of chemoattractant effect expected when the betamethasone concentration is extrapolated to infinite concentration and ID_{50} is the concentration of betamethasone that will produce $0.5 \times \%I_{max}$. The inhibition described by these curves was highly significant ($p < 0.001$) using nonlinear regression analysis.

RESULTS

Betamethasone caused a significant dose-dependent inhibition of PMN motility in all 18 neonates studied. A summary of these data is shown in Figure 1 and Table 1. The configurations of the inhibition curves most closely approximated rectangular hyperbola saturation curves. The $\%I_{max} \pm 1$ SE of the chemotaxis curve is 49.7 ± 2.6, $r = 0.626$, df = 61, $p < 0.001$, with an $ID_{50} \pm 1$ SE of 0.0134 ± 0.0024 μg/ml while the $\%I_{max} \pm 1$ SE of the random migration curve is 22.1 ± 1.1, $r = 0.699$, df = 45, $p < 0.001$ with an $ID_{50} \pm 1$ SE of 0.00374 ± 0.00094 μg/ml. There was significant impairment in PMN random migration and chemotaxis at all betamethasone concentrations tested ($p < 0.01$) including those reported in cord blood (0.01–0.02 μg/ml) following prenatal maternal betamethasone administration. The inhibitory effect of betamethasone was unaffected by saline washing of PMNs preincubated with betamethasone at 0.01 and 0.02 μg/ml. PMNs from 14 adults were incubated in betamethasone at the same concentrations from 0.005 to 10 μg/ml as those used in the neonatal PMN studies. Betamethasone caused no inhibition or stimulation of adult PMN motility up to concentrations of 10 μg/ml.

DISCUSSION

It was first noted by Liggins and Howie in 1972 (17) that antenatal administration of glucocorticoids to mothers of preterm infants could accelerate lung maturation. Subsequent clinical trials have confirmed these observations (12, 13, 18). According to current obstetrical practice, many women in spontaneous premature labor receive two intramuscular injections of a suspension containing 6 mg betamethasone acetate and 6 mg betamethasone phosphate (Celestone Soluspan) 24 h apart. Ballard *et al.* (19) determined the amount of betamethasone transferred to an infant following maternal administration by measuring both maternal and cord blood levels of betamethasone. Peak cord blood levels achieved were 0.01 to 0.02 μg/ml with the standard 12-mg dose of betamethasone. The results of the present study demonstrate that these levels markedly inhibit neonatal PMN motility following *in vitro* exposure for less than 2 h. Since Ballard *et al.* (19) noted that these peak levels persisted for at least the first 4 h after betamethasone administration, it is unlikely that the *in vivo* response of PMNs to betamethasone would differ from our *in vitro* observations due to rapid *in vivo* degradation of betamethasone.

PMNs are critical components of the acute inflammatory response to bacterial infection. PMN motility is diminished in healthy term neonates and even further impaired in healthy and stressed premature infants (3–5, 11, 20). Thus the marked inhibitory effect of betamethasone on PMN function noted in this study might be expected to have significant consequences in neonates. It is the premature infant who is most likely to receive betamethasone since the drug is given to mothers who are delivering prematurely in order to accelerate fetal lung maturity (12). In some nurseries steroids are administered directly to the newborn as adjunctive therapy for lung disease or to decrease laryngeal edema following endotracheal tube extubation. In these settings serum levels of corticosteroids may be exceedingly high

Table 1. *Effect of betamethasone on PMN motility from 18 newborn infants**

Betamethasone concentration (μg/ml)	0.005	0.0075	0.01	0.02	0.1	1	10
% change (±1 SE) in mean PMN random migration value compared with control	−12.6 ± 3.0	−13.4 ± 2.9	−15.0 ± 0.8	−21.7 ± 1.6	−21.9 ± 2.0	−20.6 ± 1.1	−39 ± 4.5
% change (±1 SE) in mean PMN chemotactic value compared with control	−11.7 ± 3.1	−18.8 ± 2.7	−23.5 ± 3.0	−28.4 ± 2.7	−42.2 ± 4.5	−49 ± 7.0	−51.7 ± 7.7

* PMNs from 18 neonates were separated from whole blood and in each case aliquots of 1 ml containing 5×10^6 PMNs were incubated in buffer solution or in one of several concentrations of betamethasone for 30 min. PMN random migration and chemotaxis were determined. When the mean value for the simultaneously run buffer controls for each betamethasone concentration are combined, the total mean buffer control values are 52.1 ± 13.7 μm for random migration and 92.4 ± 16.8 μm for chemotaxis. The value of random migration for a betamethasone concentration of 10 μg/ml was significantly greater than that predicted by nonlinear regression analysis and was not included in the regression calculations. A significant ($p < 0.01$) decrease in both PMN random migration and chemotaxis was noted in PMNs incubated in all of the betamethasone concentrations compared with controls using analysis of variance (Dunnett's test).

and would be expected to have an even greater inhibiting effect than those following maternal corticosteroid administration based on the data from this study. The results of this study may also help explain why PMN chemotaxis is impaired in healthy neonates and further impaired in stressed neonates such as those with RDS. The peak neonatal betamethasone levels following antenatal betamethasone administration which were found to significantly inhibit PMN motility are comparable to the 3-fold increase in glucocorticoid levels found in premature infants with RDS and double the 1.5-fold increase found in premature infants who are delivered after premature rupture of membranes.

Mothers who have received antenatal steroid administration consisting of a maximum of six injections of dexamethasone over 48 h have been shown to be at increased risk for infection, especially with ruptured membranes for more than 48 h (21). There are conflicting reports as to whether the infants of these mothers share this risk. The Collaborative Group on Antenatal Steroid Therapy showed a nonsignificant decrease in incidence of infection in neonates born to mothers receiving corticosteroids. However, cultures were only obtained in symptomatic infants (13). These infants also had a lower incidence of lung disease and its attendant complications. In two other large studies, a greater number of neonatal infections was noted with maternal corticosteroid administration but the differences from control subjects were not statistically significant (21, 22). Lazzarin *et al.* (14, 15) in two separate studies have reported a significant increase in the infection rate in both mothers and premature infants and a significant decrease in neonatal PMN chemotaxis following antenatal steroid administration for lung maturation.

Several studies have documented the inhibitory effect of corticosteroid compounds on several aspects of PMN function including PMN adherence, phagocytosis, and intracellular killing (23–26). Previous studies of the corticosteroid effect on PMN motility have been performed in adults. The results have varied with some finding inhibition (27–29) of PMN chemotaxis and others finding no effect or stimulation (27, 30). In one study in which adult PMNs were incubated in betamethasone at concentrations similar to those used in our study, betamethasone was found to inhibit PMN motility (29). The distal surface method of PMN chemotaxis analysis was used, however, which is subject to a number of methodological problems. These include: 1) cell detachment at the distal surface which is variable, related to time of incubation, and indirectly related to PMN adherence, 2) distortions of the actual response because of heterogeneity of PMN chemotaxis so that PMNs which arrive close to the distal surface are not counted, or in the case of long incubation times, rapid PMNs are not counted because they detach from the distal surface and, 3) variability in counts due to the nonuniformity of the number and distribution of PMNs at the distal surface (16). Thus, discordant results in studies of corticosteroid effects on adult PMN chemotaxis are probably due to the wide variety of type and dosage of corticosteroids examined and the use of different PMN motility assays. The reason for the apparent increased sensitivity of neonatal PMN motility compared with that of adults to the inhibiting effects of corticosteroids is unknown and is a subject of future investigation.

In summary, data from this study indicate that betamethasone inhibits PMN random migration and chemotaxis in neonates but not in adults. These results suggest that corticosteroid administration to pregnant women or their newborn infants may be a contributing factor to the morbidity and mortality of bacterial infections in neonates. In addition, it is possible that differences in corticosteroid effect on PMN motility between neonates and adults may be exploited to further dissect the mechanism of decreased PMN motility in newborn infants.

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