PRENATAL HYDROCORTISONE ADMINISTRATION AND LYMPHOCYTE RESPONSE IN THE NEONATAL RABBITS

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The influence of hydrocortisone to lymphocyte response against PHA stimulation was studied in neonatal rabbits. Prenatally, six pregnant rabbits were given hydrocortisone and seven control pregnant rabbits were given physiological saline at the 28th, 29th and 30th day of gestation. After delivery, lymphocytes of the neonates were tested within 48 hours.

The stimulation index after the lymphocytes were cultered with PHA of 1:100 was 11.15 ± 2.14 in saline-treated control, versus 2.43 ± 1.0 in the steroid-treated group ($p < 0.005$). Similar difference in S.I. was also found with PHA 1:1000, 9.56 ± 2.84 in saline versus 1.54 ± 0.13 in the steroid group ($p < 0.02$).

Postnatally, neonates were divided into two groups, one was given hydrocortisone, the other saline on the 1st, 2nd and 3rd day of age. The neonates were sacrificed at 72-120 hours of age and the blood collected for lymphocyte studies.

The stimulation index in the treatment neonates with PHA was significantly depressed in the steroid-treated group when the results were compared with the control group. Steroid when administered prenatally or postnatally, suppresses lymphocyte function in the neonatal rabbits.

INTRODUCTION

Corticosteroids have widely been used in the treatment of inflammatory conditions (Skinner and Schwartz, 1974) and also as an immunosuppressive agent, notably in the transplantation (Koutz, 1973). The inhibitory effect of corticosteroids upon lymphocyte response *in vivo* has been known since Dougherty (1953) reported in 1952. To dermine the effect of drugs to immune system test for lymphocyte responsiveness to mitogens, *in vitro* has commonly been used as an immunological monitoring and the drugs were found to be suppress lymphocyte activation (Nowell, 1961).

The administration of corticosteroids to fetuses in experimental animals were found to be an accelerated maturation of pulmonary surfactant (De Lemos et al., 1970; De Lemos and McLaughlin, 1973; Motoyama et al., 1971). There is a great concern regarding the adverse effects of the corticosteroids on the growing fetus. Treatment of infant rats with a single injection of methylprednisone resulted in alterations in myelination with the immaturation of the brain and the normal increase in cell number was severely inhibited during the period of cortisol treatment (Cohen, 1972; Cotterrel et al., 1972; Cumbinas et al., 1973). Suppression of

thymic function following postnatal steroid therapy has been reported. Intravascular injection of methylprednisone is found to prolong the average survival of the grafts in rabbits and human (Coburg et al., 1970). Furthermore, corticosteroid administration in man has been demonstrated to cause a transient depletion of lymphocytes, predominately in the thymus-derived (T) cell population (Fanci and Dale, 1974). The purpose of this study was to investigate the effect of prenatal administration of corticosteroids on the immune system as a lymphocyte function in the rabbit neonates, and the results were compared with the effect on postnatal rabbits.

MATERIALS AND METHODS

The response of lymphocytes to PHA stimulation was used to assess function in two groups of neonatal rabbits: one prenatally-treated and the other postnatally-treated.

Prenatal Study:

Thirteen pregnant albino New Zealand rabbits with timed mating were used. Six were treated with hydrocortisone sodium succinate (Solucorted, Upjohn Co., Kalamazoo, Michigan) 10 mg/kg (0.5 ml) and seven with 0.5 ml of normal saline at 28th, 29th and 30th days of gestation, and they were allowed to deliver spontaneously.

Blood for lymphocyte studies was collected in heparinized tubes within 48 hours of age. Because of the small quantity of blood obtained from each newborn, blood from all neonates in the litter was pooled for the study.

Postnatal Study:

Seven litters born to pregnant rabbits after term gestation were used.

Half of the neonates in the litter were given hydrocortisone 20 mg/kg, and the remaining neonates were injected with 0.5 ml saline subcutaneously at 1, 2 and 3 days of age. Neonates were sacrificed at 72-120 hours of age and blood collected in heparinized tubes by decapitation. All the seven neonates receiving steroid were included in the study.

Preparation and Culture of Lymphocyte Suspension:

Mononuclear cells were obtained by Hypaque-Ficoll density gradient centrifugation (Boyum, 1968). The cells were washed twice in RPMI media (RPMI-1640 with glutamine without antibiotic, Grand Island Biological Company (GIBCO), Grand Island, N. Y. 14072) and suspended in RPMI media containing 10% fetal calf serum (GIBCO), 100 μ /ml of penicillin (E. R. Squibb and Sons Inc., Princeton, N. J.) and 100 μ g/ml of streptomycin sulphate (Pfizer Laboratories Division, New York). Cultures were done in microtiter plates (Cook Laboratory Products, Cook Laboratory Products, Cook Engineering Co., Alexandria, VA.). Each well contained 0.1 ml of cells in a concentration of 1×10^6 lymphocytes/ml and 0.1 ml of either RPMI media with fetal calf serum of mitogens in different concentrations. Phytohemagglutinin (PHA, MR68, Lot K 4402, The Wellcome Research Laboratories, Beckenham, Kent, England) in concentration of 1:100 and 1:1000 was used as a mitogenic stimulant. Triple cultures were incubated at 37°C in 5% CO₂ in air, and 100% humidity for 72 hours. Four hours before harvesting, 0.4 μ ci of tritiated thymidine (6.7 μ ci/mN, New England Nuclear, Boston, Mass.) was added to each well. The cells were collected from the wells onto a glass fiber filter (Grade 93AH Reeve Angel, Lot No. 5016, Whatman Inc.,

Dedham, Mass., CHAP 100) and placed in 10 ml of aquasol (New England Nuclear). Radioactivity was measured in a liquid scintillation counter (Packard Tri-Carb Liquid Scintillation Spectrometer, Model 3003). The arithmetic mean of counts per minute (cpm) of triplate cultures was determined and stimulation index (S.I.) was expressed as a ratio of count per minute in stimulated and unstimulated cultures. Stimulation index was compared between the control and steroid group. The student t-test was used to measure the statistical significance.

RESULTS

In Table I, data of the prenatally-treated neonates is shown. The mean age at study in saline treated group and steroid group was two days. The S.I. when cultured with PHA of 1:100 dilution was 11.15 ± 2.14 in saline-treated, versus 2.43 ± 1.0 in the steroid-treated group. There was a significant difference between the two groups ($p < 0.005$). Similar difference in stimulation index was found with PHA 1:1000 dilution, 9.56 ± 2.84 in saline versus 1.54 ± 0.13 in steroid group, $p < 0.02$.

TABLE 1

Results of lymphocyte stimulation with and without PHA comparing between prenatal control and steroid-treated groups

Prenatal Control Group						
No. Rabbit	Age at Expt. (hours)	CPM (Unstimulated)	CPM (PHA1 : 100)	S. I.	CPM (PHA1 : 1000)	S. I.
1	96	1694.4	9033.8	5.33	6046.5	3.56
2	23	1944.0	5873.7	3.02	5556.4	2.83
3	1	88.4	1365.6	15.44	1163.6	13.15
4	48	119.4	1639.2	13.74	558.6	4.68
5	48	21.8	350.6	15.90	288.3	13.22
6	48	18.6	313.6	16.83	437.9	23.50
7	48	28.8	225.1	7.82	225.1	6.02
Mean	44.6	559.37	2685.92	11.15	2039.5	9.56
$\pm$ S.D.	± 10.9	± 326.7	± 1200.68	± 2.14	± 981.79	± 2.84
Prenatally Steroid-Treated Group						
No. Rabbit	Age at Expt. (hours)	CPM (Unstimulated)	CPM (PHA1 : 100)	S. I.	CPM (PHA1 : 1000)	S. I.
1	48	251.7	574.4	2.28	285.5	1.13
2	48	437.5	437.5	0.88	643.0	1.30
3	48	7275.4	13490.1	1.85	10338.6	1.42
4	48	27.0	31.3	1.16	37.0	1.37
5	48	441.9	3371.1	7.62	1209.0	2.73
6	48	420.0	356.8	0.86	553.0	1.29
Mean	48	1484.61	3043.58	2.43	1015.12	1.54
$\pm$ S.D.		± 1164.7	± 2156.34	± 1.06	± 536.0	± 0.13

TABLE 2
Comparison of lymphocyte stimulation between control and steroid-treated groups with and without PHA

Postnatal Control Group						
No. Rabbit	Age at Expt. (hours)	CPM (Unstimulated)	CPM (PHA1 : 100)	S.I.	CPM (PHA1 : 1000)	S. I.
1	96	14.0	20.9	1.48	23.67	1.68
2	96	19.1	105.3	5.54	179.26	9.43
3	96	21.5	55.6	2.58	34.18	1.59
4	120	34.8	483.3	13.80	412.20	11.80
5	96	42.5	164.7	3.87	343.05	8.10
Mean	100.8	26.44	165.9	5.45	198.47	6.52
±S.D.	±4.79	±5.29	±83.17	±2.19	±79.11	±2.15

Postnatally Steroid-Treated Group						
No. Rabbit	Age at Expt. (hours)	CPM (Unstimulated)	CPM (PHA1 : 100)	S.I.	CPM (PHA1 : 1000)	S. I.
1	120	120.2	97.6	0.81	52.3	0.43
2	72	23.3	36.6	1.57	12.3	0.52
3	96	69.2	86.6	1.25	57.5	0.83
4	96	32.4	25.4	0.78	23.5	0.72
5	96	11.2	26.2	2.34	14.1	1.26
6	96	17.0	12.2	0.71	25.3	1.48
7	96	21.3	20.7	0.96	81.2	3.79
Mean	99.42	42.1	43.64	1.20	38.1	1.29
±S.D.	±6.27	±14.9	±12.90	±0.22	±9.84	±0.43

TABLE 3
Statistical analysis of stimulation index between control and steroid-treated groups

Prenatal	Saline-treated Mean±S.D.	Steroid-treated Mean±S.D.	p
PHA (1 : 100)	11.15±2.14	2.43±1.06	<0.005
PHA (1 : 1000)	9.56±2.84	1.54±0.13	<0.02
p	n. s.	n. s.	

Postnatal	Saline-treated Mean±S.D.	Steroid-treated Mean±S.D.	p
PHA (1 : 100)	5.45±2.19	1.20±0.22	<0.05
PHA (1 : 1000)	6.52±2.19	1.20±0.22	<0.05
p	n. s.	n. s.	

n.s. Not Significant

The data of postnatally treated neonates is shown in Table II. In both groups, the studies were conducted around 4 days of postnatal age. The S.I. both with PHA (1:100) and PHA (1:1000) was significantly depressed in the steroid group, $p < 0.005$. Table III shows the summary of the results in both prenatal and postnatal groups.

DISCUSSION

There is convincing evidence of the beneficial effect of prenatal steroid administration in accelerating the lung maturation (DeLemos, et al., 1970; DeLemos and McLaughlin, 1973; Motoyama, et al., 1971). However, there is a controversy regarding the side effects of such treatment of the fetus and the neonate. In animals, decreased fetal cellular growth of lungs, brain and thymus have been reported following corticosteroid therapy on the fetus (Carson, et al., 1973; Cohen, 1972; Cotterel, et al., 1972; Cumbinas, et al., 1973; DeSouza and Adlard, 1973). Corticosteroid therapy is also known to have a number of immunosuppressive effects such as: decreased antibody production (Butler and Rossen, 1973; Greenberg and Dimitrov, 1976), lymphopenia (Boyum, 1968; Esteban, 1968; Fauci, 1976) and suppression of cutaneous delayed hypersensitivity (Bovorknitti et al., 1960). Fauci (1976) has demonstrated temporary lymphopenia and decreased response of human lymphocytes to mitogen even with a single dose of hydrocortisone. Furthermore, *in vitro* studies, inhibition of mitogenesis was seen following the addition of corticosteroid to lymphocyte culture (Yu, et al., 1974). These findings indicate the need for study of effects on corticosteroid therapy on the immune response of the fetus. Fitzharding et al. (1974), re-

ported normal immunocompetence at one year of age in infants who received corticosteroid at 24 hours of age. No such data is available in infants who received steroid therapy prenatally. In this study, stimulation of lymphocyte with PHA as an index of lymphocyte function was studied in both prenatally and postnatally treated neonatal rabbits. In steroid treated group, stimulation index was significantly impaired. Prenatal or postnatal administration did not show statistical difference in impairment. It should be noted that the steroid dose in the postnatal group was twice as much as the dose administered prenatally, which explains the apparent increased suppression of stimulation in the postnatal group (1.20 versus 2.43 at the dilution of PHA 1:100). The stimulation index between the prenatal and postnatal saline group showed no statistical difference although there was a trend towards the decreased mitogenic response in the postnatal group. We speculate that the stress of injection in the postnatal group itself may have played a role in this impairment.

This study demonstrated that steroid when administered prenatally or postnatally, suppresses lymphocyte function in the rabbit neonate. Persistence of immunosuppressive effect after administration of corticosteroid in the neonate needs a further study, but these findings could be of great significance in a preterm human neonate in whom administration of corticosteroids will suppress further, the already impaired cellular immunity.

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