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LOW LEVELS OF GLUCOCORTICOID BINDING SITES IN CIRCULATING LYMPHOCYTES OF PREMATURE INFANTS SUFFERING FROM HYALINE MEMBRANE DISEASE

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Summary—The number and affinity of glucocorticoid binding sites in lymphocytes of newborns and prematures were determined by a whole cell [^{3}H]dexamethasone binding assay. The average binding capacities were as follows: 1758 ± 245 binding sites/cell in cord blood, 2758 ± 307 binding sites/cell in mature newborns (K_d: $6{,}23 \times 10^{-9}$ M), and 2031 ± 330 binding sites/cell in prematures. No specific binding was measurable in several cases. All the prematures, who did not display a measurable glucocorticoid binding capacity, suffered from a serious hyaline membrane disease (HMD), they died on 3.5–4 days of life. HMD diagnosis was established in 12 cases *in toto*. The number of glucocorticoid binding sites was significantly smaller in this group (639 ± 216 per cell) than in the case of the rest of prematures (2959 ± 404 per cell, $P < 0.001$). Our results suggest that lack of glucocorticoid receptors may be one of the causes of the HMD.

INTRODUCTION

Recently, it has been established that several mammalian tissues display a significant number of glucocorticoid receptors [1–5]. Receptors have been analyzed also in a number of diseases [3,6] and correlations have been found in several cases between receptor number and clinical outcome [1].

The HMD is a major cause of neonatal mortality, especially in premature infants. Hyaline membrane disease (HMD) or idiopathic respiratory distress syndrome is a clinical manifestation of lung immaturity in the newborn infant [7] characterized by atelectasis, decreased lung compliance and right-to-left shunting. The best known feature in pathogenesis of HMD is the primary or secondary deficiency of the surfactant synthesis. Clinically, this impairment in gas exchange is manifested in tachypnea, intercostal and substernal retractions during inspiration, expiratory grunting, and brief periods of apnea. Severely affected infants develop marked hypoxia, hypercarbia, respiratory and metabolic acidosis, and cyanosis which may result in the need for mechanical ventilation. HMD is classified into four stages according to the seriousness of diseases [8].

Glucocorticoids are known to accelerate maturation of the lungs and they have been administered as prenatal therapy in recent years. Since the employment of this therapy, frequency of HMD and mortality of patients have significantly been reduced, still it could not eliminate HMD until now [9].

Our goal was to compare the number of glucocorticoid receptors in peripheral lymphocytes of healthy newborns, of prematures, and of babies suffering from HMD. We hoped that, based on this kind of data, we would be able to tell in retrospect why prenatal glucocorticoid therapy was successful in some cases, but not in others, in prevention of HMD.

EXPERIMENTAL

Our patients were 20 mature newborns (gestational age 39.6 ± 1.3 weeks, birth weight 3420 ± 170 g) and 30 prematures (gestational age 32.42 ± 2.4 weeks, birth weight 1706 ± 52.76 g). Blood samples were taken from peripheral vein, in some cases through umbilical catheter for technical reasons, 48–72 h after birth, at 8 a.m. Clotting was inhibited by heparin. Umbilical blood samples were also taken in ten cases when normal babies were born in deliveries without complications. Fifteen out of the thirty prematures were treated with steroid in the prenatal period as follows: Mothers were given 15 mg oradexon i.m. once, 42 ± 1.7 h before delivery, on average. Deliveries were passed all *per vias naturales* with the exceptions of two section Caesarea cases. The twenty newborns were healthy, out of the thirty prematures 5 were sympton free, 12 suffered from HMD, 4 from bronchopneumonia, 2 from wet lung syndrome, 1 from hyperviscosity syndrome, and in 6 cases hyperbilirubinemia (exchange transfusion was needed in case of two babies) as shown in Table 1.

The diagnosis of HMD I–IV was established on the basis of clinical picture, X-ray findings, blood gases, pH and pathology.

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Table 1. Glucocorticoid binding sites in lymphocytes of premature infants

No.	Sex	Gestational age (weeks)	Weight at birth	Diagnosis	Binding sites/cell	Mean ± SEM
1	F	34	1850	HMD I–II	2304	
2	F	36	2130	HMD II	1324	
3	F	29	1520	HMD II	920	
4	M*	33	2005	HMD III–IV	Not measurable	
5	M*	31	1300	HMD III–IV	Not measurable	
6	F*	31	1300	HMD III–IV	Not measurable	
7	M*	32	1920	HMD III–IV	Not measurable	1466 ± 347
8	M*	31	1410	HMD IV	Not measurable	
9	M	35	1650	Bronchopneumonia	2772	
10	F	33	1900	Bronchopneumonia	2700	
11	F	34	2300	Hyperbilirubinemia	1029	
12	M	34	1870	Hyperbilirubinemia	3812	
13	F	33	1710	Hyperbilirubinemia	2810	
14	F	34	1750	Prematurity	1195	
15	F	36	2050	Prematurity	3132	
16	F	34	1800	HMD II	192	
17	M	26–27	1300	HMD II	734	
18	F	29	1320	HMD II	820	
19	M*	32	1400	HMD IV	1382	
20	M	36	1950	Bronchopneumonia	1612	
21	F	33	1710	Bronchopneumonia	2890	
22	F	31	1550	Hyperbilirubinemia	1122	
23	M	32	1950	Hyperbilirubinemia	4874	2596 ± 535
24	F	34	1730	Hyperbilirubinemia	5390	
25	M	31	1600	Wet lung syndrome	6528	
26	M	35	1850	Wet lung syndrome	5860	
27	M	34	1950	Prematurity	1611	
28	F	28	1290	Prematurity	907	
29	M	32	1680	Prematurity	2422	
30	F	29	1440	Hyperviscosity syndrome		2600

Glucocorticoid binding sites in lymphocytes of prematures was determined as described.
Nos 1–15: no prenatal steroid administration.
Nos 16–30: mothers received 15 mg oradexon i.m. once 47 ± 2.3 h before delivery.
Patients Nos 11 and 23 received exchange transfusion.
* = died.

Chemicals

[1,2-^{3}H]Dexamethasone (sp. act. 1,48 TBq/mol) was obtained from the Radiochemical Centre Amersham (U.K.), unlabelled dexamethasone from Sigma, oradexon from Organon. All other chemicals were obtained from Reanal, Budapest, Hungary.

Determination of dexamethasone binding in whole cells

This was done essentially as described in [10]. Blood was separated by Ficoll–Uromiro density gradient centrifugation [11], then washed with Hank's medium. To 100 μl of cell suspension, containing 1–5 × 10^6 cells, was added 100 μl of [^{3}H]dexamethasone diluted in the same medium. To determine non-specific binding, identical aliquots were incubated in the presence of 5 × 10^{-6} M unlabelled dexamethasone. Multiple concentrations 2.5–50 nM of radioactive dexamethasone were used to construct complete binding curves. All incubations were performed at 37°C for 30 min with continuous shaking. This time was found in pilot experiments long enough to achieve steady state. At the end of the incubation period, samples were centrifuged at 800 *g* for 10 min in the cold and washed three times with 5 ml ice cold Hank's solution. The final pellet was resuspended in 5 ml of scintillation fluid containing 33% (v/v) Triton X-100 in toluene solution. Specific binding was taken to be the difference in radioactivity of samples incubated with and without unlabelled dexamethasone. Binding sites per cell and dissociation constants were obtained from Scatchard analysis of the data [12]. The radioactivity was measured with Nuclear Chicago ISOCAP 300 radiospectrofluorimeter.

RESULTS

The number of glucocorticoid binding sites per cell was the lowest in lymphocytes obtained from umbilical blood of mature healthy newborns: 1758 ± 245/cell. The average of glucocorticoid binding sites of peripheral lymphocytes of mature newborns amounted to 2758 ± 307 sites/cell. Dissociation constant, as obtained from Scatchard analysis of the data, was 6.23 × 10^{-9} M in this particular experiment. The average of glucocorticoid binding sites of peripheral lymphocytes of prematures was 2031 ± 330 sites/cell. Within this, we could find no correlation between receptor number and gestational age, birth weight or sex. High values were found in case of prematures treated with steroid prenatally: 2596 ± 535 sites/cell, whereas lymphocytes of prematures who had not been treated with steroid had only 1466 ± 347 sites/cell. HMD diagnosis was established in 12 cases. Glucocorticoid binding site within this group was 639 ± 216 sites/cell. The rest of pre-

matures (18 cases, some of them suffering from diseases other than HMD) had significantly higher glucocorticoid binding sites per cell: 2959 ± 404 [$P < 0.001$], (Table 1).

Specific binding was not measurable in several cases. All the prematures who did not display measurable glucocorticoid binding sites, suffered from a serious HMD, they died on 3.5–4 days of life. Their section findings proved the clinical diagnosis.

DISCUSSION

Ballard and Giannopoulos[1, 13–15] examined extensively the glucocorticoid binding in fetal lung tissues. The presence of high affinity dexamethasone binding sites has been demonstrated in cytoplasmic extracts from lungs of normal human neonates, but not in lungs of prematures with HMD. In spite of the success of prenatal glucocorticoid treatment in HMD therapy, it cannot be used in every case, because of well-known maternal side-effects. On the other hand, sometimes HMD of various seriousness develops, in spite of prenatal maternal steroid administration. Steroid receptors of lung tissues of living human newborns or prematures can not be determined, since no tissue samples can be taken. However, according to Okret's[16] description, glucocorticoid receptors in different tissues of the same individual are immunologically similar. Maybe their expression is also related in certain cases. For this reason, we examined glucocorticoid binding in peripheral lymphocytes. The average of glucocorticoid binding sites in peripheral lymphocytes of mature healthy newborns was found 2758 ± 307 sites/cell (K_d: 6.23×10^{-9} M) and a similar number was observed in lymphocytes of premature infants treated prenatally with dexamethasone. We are not aware of any previous data in the literature on glucocorticoid binding in lymphocytes of newborn and premature infants. According to our results, receptor content of the lymphocytes of a newborn is slightly lower and the affinity for dexamethasone is about the same as those found in adults [17–20].

We could find no correlation between receptor number and gestational age, birth weight or sex. Ballard *et al.*[13] could not demonstrate specific binding sites in the lungs of prematures who died of HMD. In agreement with this, no specific dexamethasone binding activity could be measured in our five serious HMD cases with fatal outcome. One could argue that elevated endogenous glucocorticoid levels may mask the cellular receptors and an artificially small number of binding sites would be observed as a consequence of that. Very likely, however, this is not the case. First, endogenous glucocorticoids were mostly removed from the cells by the washing procedure, so that they did not interfere with our assay. Second, in a number of cases, serum glucocorticoid levels were determined in parallel with the measurement of the binding sites (data not shown). No correlation whatsoever was found between glucocorticoid level and number of lymphocyte glucocorticoid binding sites of the same individual. Thus, a possible explanation of our finding is that the lymphocytes of the prematures who developed serious HMD either contained no glucocorticoid receptors, or an inactive form of the receptor. HMD diagnosis was established in 12 cases *in toto*. The number of glucocorticoid binding sites was significantly smaller in this group than in the case of the rest of prematures. Perhaps a defect in hormone binding may be a reason of the lack of surfactant in the lungs and in turn of the occurrence of HMD.

According to our opinion, glucocorticoid binding capacity of lymphocytes may reflect glucocorticoid binding in the lung tissue and may possibly be of diagnostic value. A significant extension of our data basis is necessary, however, to prove this hypothesis.

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