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Effect of Antenatal Dexamethasone on Postnatal Alveolar Diameter

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ABSTRACT

Dexamethasone (DEX) is a potent synthetic glucocorticoid. It is given to women at risk of delivering prematurely in order to promote maturation of fetal lungs. **Objectives:** To evaluate the effects of DEX given to the albino rats during different stages of intrauterine lung development on the postnatal alveolar diameter. **Materials and Methods:** 40 dams were randomly separated into five equal groups A, B, C, D and E. DEX (injection Decadron) was given in a dose of 0.2 mg/kg body weight to the dams of group B on 11, 12 and 13 gd (embryonic stage), to group C on 16, 17 and 18 gd (pseudoglandular stage), to group D on 19 and 20 gd (canalicular stage) and to group E on 21 gd (saccular stage). Group A dams didn't recieve any drug. After normal delivery of the dams of all groups, the sample size was obtained by collecting 24 pups from each group which were labeled as B1, C1, D1 & E1 respectively. Pups taken from group A were considered as control A1. Pups of all groups were allowed to grow upto 28 postnatal day. Right lungs were collected from the pups of each group after proper fixation. Serial 3µm sections were cut and stained with hematoxylin/eosin stains for detailed histological study of alveoli. **Results:** The pups of group B1 showed results similar to those of control group. The pups of group C1 and E1 showed increased mean diameter of alveoli ($P<0.001$). The pups of group D1 showed highest mean diameter of alveoli with P-value <0.001. **Conclusion:** DEX has direct influence on developing lungs especially alveolar diameter even by little dose administration in prenatal stages of lung development.

Key words: Dexamethasone, Albino rat, alveoli, development.

INTRODUCTION

Glucocorticoid drugs are the synthetic compounds which have anti-inflammatory effects like natural glucocorticoids. Dexamethasone (DEX) is the long acting primary synthetic glucocorticoid[1]. It is used in those cases of gestation which have risks of prematurity[2]. Pre-natal administration of the drug improves the development of certain organs of the fetus, which in return increases the probability of premature survival[3]. It is a type of medical intervention that improves health care and produces considerable cost saving[4].

The dosing regimen of four 6-mg doses of DEX administered every 12 hrs reduces the incidence of respiratory distress syndrome (RDS) by only 50%[5]. For further reduction of RDS, multiple doses were administered to the women at risk for preterm delivery, which resulted in cardiovascular, neuronal and developmental defects in the fetus instead of adding any benefit[6].

Pua et al. cited in their work that Liggins and Howie published the first study demonstrating the value of maternal antenatal corticosteroids (ACS) to decrease the incidence of respiratory distress syndrome in 1972. The consensus statement of the American National Institute of Health now recommends administration of ACS to all patients at risk of preterm delivery prior to 34 weeks of gestation[7]. These recommended four doses of prenatal DEX therapy are equivalent to the physiological stress response experienced by premature infants and it leads to almost more than

75% receptor occupancy in the target cells.[6] The exact sites of glucocorticoid action in the fetal lung remain uncertain, however during pseudoglandular phase of mouse lung development, radiolabeled DEX binding mostly occurs in the interstitial mesenchyme, whereas during late gestation, increasing GR content is noticed in the distal airway epithelium[8].

This study was designed to evaluate the effects of DEX given to the albino rat during different stages of intrauterine lung development on postnatal alveolar diameter.

MATERIALS AND METHODS

Forty female albino rats of Sprague-dawley strain (weighing about 250-300 g) were obtained from Veterinary Research Institute, Lahore. They were kept in cages for 15 days in the animal house for the purpose of acclimatization. A 12 hours light / dark cycle was maintained. The animals were allowed free access to food and water. After conception, female rats were randomly divided into five equal groups labeled as A, B, C, D and E. DEX in injectable form was given to the rats subcutaneously. The dose schedule was as follows:

Group A

It was the control group containing 8 dams which did not receive any drug.

Group B

It contained 8 dams which were given 0.2 mg/kg body weight of DEX subcutaneously on 11th, 12th and 13th day of gestation (embryonic stage of lung development) [9].

Group C

It contained 8 dams which were given 0.2 mg/kg body weight of DEX subcutaneously on 16th, 17th and 18th day of gestation (pseudoglandular stage of lung development) [9].

Group D

It contained 8 dams which were given 0.2 mg/kg body weight of DEX subcutaneously on 19th and 20th day of gestation (canalicular stage of lung development) [9].

Group E

It contained 8 dams which were given 0.2 mg/kg body weight of DEX subcutaneously on 21st day of gestation (saccular stage of lung development) [9].

The dams of each group (A, B, C, D and E) were allowed to deliver normally. 24 pups (12 males and 12 females) from each group were randomly selected. These pups were labeled as A1, B1, C1, D1 and E1. These pups were allowed to grow up to 28 days of age, because at that time formation of lung alveoli was completed in rats[10].

Dissection and fixation of lung

On 28th day, rats of group A1, B1, C1, D1 and E1 were weighed again and anesthetized with pentobarbital sodium 90 mg/kg intraperitoneally[11]. A bilateral pneumothorax was produced by puncturing the diaphragm from its abdominal surface to collapse the lungs. The trachea was cannulated and the cannula was tied firmly in place[12]. The rat was killed by infusing 1.5ml neutral phosphate buffered formaldehyde 4% into the right bronchus[13]. The right bronchus was ligated and the lungs were monitored carefully for the initial 10 min, and only those lungs, which did not leak during this time, were used for fixation and embedding. After this internal fixation, the right sided lung was removed from the chest cavity. It was weighed and external morphological study was carried out. The lungs were kept in the same neutral phosphate buffered formaldehyde 4% solution for 2 hours at 0-4°C[12]. All four lobes of right lung were placed into individual cassettes and processed in automatic tissue processor then embedded in paraffin. The central portions of the blocks were sectioned at 3 micron intervals with rotary microtome. The sections were mounted on glass slides, deparaffinized, hydrated and stained with hematoxylin & eosin. Diameter of alveoli was measured using 10x lens of light microscope. Ten alveoli having well defined boundaries and similar sizes were selected on each slide. Maximum diameters in two directions were taken. Diameters of group A1 were taken as reference. All other experimental groups were compared with the control group.

Statistical analysis

Data was analysed by SPSS Version 16. Diameter of alveoli was described by Mean±S.D and comparison between the groups were made by ANOVA. The P-value less than 0.05 was considered as statistically significant.

Table 1: Effects of DEX on alveolar diameters (µm) of control and experimental groups' off springs

Group	Gender	Mean	SD	Minimum	Maximum
A1	Female	67.27	3.10	62.25	72.50
	Male	66.88	3.22	62.50	72.50
B1	Female	65.67	3.14	61.50	70.50
	Male	65.79	2.68	62.00	70.25
C1	Female	73.33	2.72	66.00	79.00
	Male	72.46	3.63	65.00	77.25
D1	Female	76.46	5.48	70.00	82.50
	Male	75.88	4.66	68.50	81.50
E1	Female	74.04	3.76	67.50	81.25
	Male	73.42	3.82	68.00	79.50

Table 2: Comparison of alveolar diameters (µm) of rat pups of control and experimental groups (ANOVA)

Source	Sum of Squares	Df	Mean Square	F	P-value
Group	2323.783	4	580.946	42.053	<0.001**
Gender	22.751	1	22.751	1.647	0.202++
Group × Gender	35.763	4	8.941	0.647	0.630++
Error	1519.599	110	13.815		
Total	3901.896	120			

Group	Group	Mean difference	Std. Error	P-value
A1	B1	0.8438	1.07295	0.934++
	C1	-6.8229	1.07295	< 0.001**
	D1	-9.5938	1.07295	< 0.001**
	E1	-8.6563	1.07295	< 0.001**
B1	C1	-7.6667	1.07295	< 0.001**
	D1	-10.4375	1.07295	< 0.001**
	E1	-9.5000	1.07295	< 0.001**
C1	D1	-2.7708	1.07295	0.081++
	E1	-1.8333	1.07295	0.433++
D1	E1	0.9375	1.07295	0.906++

× Interaction effect
** Highly significant difference (P<0.01)
++ Non significant difference (P>0.05)

RESULTS

The mean diameter of alveoli in control group A1 was calculated as 67.27±3.10µm for female pups and 66.88±3.22 µm for male pups. When comparison was made for diameter of alveoli in pups of different groups, it was observed that the Group D1 was having highest diameter, while the group B1 had the lowest. Statistically the group differences were significant with P-value <0.001 and the gender difference and the interaction difference were insignificant with p-values 0.202 and 0.630 respectively. When group-wise comparisons were made, it was observed that A1 and B1 had statistically significant differences as compare to C1, D1 and E1 (P-value <0.001). The differences between C1, D1 and E1 were statistically insignificant with each other (P-value >0.05), similarly difference between A1 and B1 was statistically insignificant with P-value 0.934 (Table 1, Figs. 1-5).

DISCUSSION

DEX is a potent synthetic member of the glucocorticoid class of steroid drugs. It accelerates the development of alveoli in the fetuses when given to the pregnant mothers during preterm labour. The effects produced by DEX persist even on 28th postnatal day. Interestingly, human and rat fetal lung glucocorticoid receptors have very similar affinity for DEX, also the half life of DEX is identical in humans and rats[6]. While comparing the anatomy of lung, we know that typical pair of human lungs contains about 700 million alveoli, producing 70m² of surface area. An adult alveolus has an average diameter of 200 micrometres[14]. While the average number of alveoli in the albino rat lungs is 20.1 million and the diameter of alveoli is about 70 micrometer[15]. We hypothesized that DEX given to pregnant female rats could effect the diameter of alveoli in the newborn pups and this change might persist upto 28th postnatal day. The results of our study showed that the diameter of alveoli was increased in group C1, D1 and E1, but group B1 was having diameter similar to the control group. Group D1 showed highest diameter of alveoli. The dams of group D1 had received dose of DEX on 19th and 20th day of pregnancy. Study done by Massaro et al (1995) showed similar results. In that study, pregnant rats were injected DEX

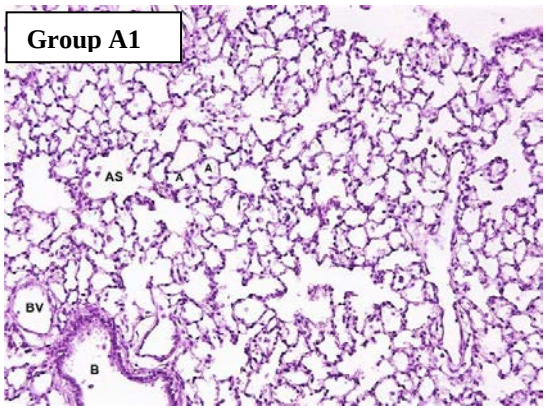

Fig. 1: Photomicrograph of fetal rat lung of Group A1 showing bronchiole (B), Alveolar sac (AS), Blood vessel (BV) and normal sized Alveoli (A). H&E x 10

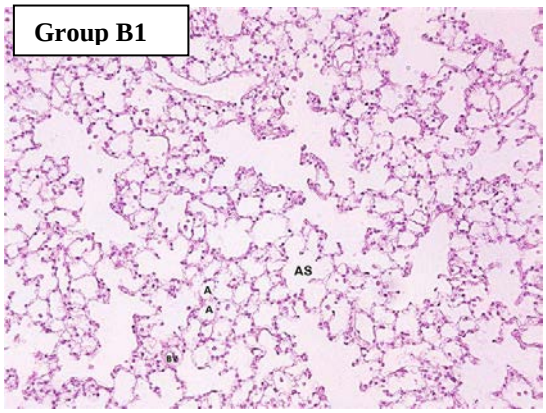

Fig. 2: Photomicrograph of fetal rat lung of Group B1 showing alveolar sac (AS) and normal sized Alveoli (A). H&E x 10

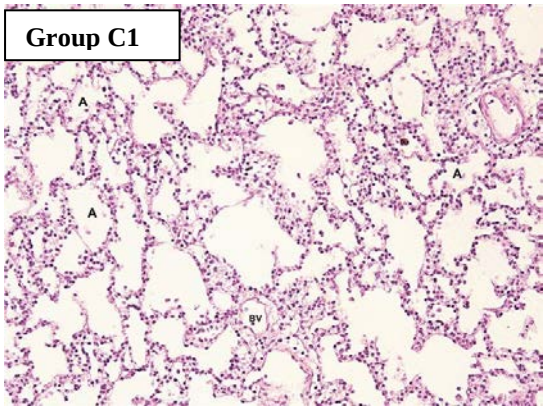

Fig. 3: Photomicrograph of fetal rat lung of Group C1 showing focal enlargement of alveoli (A). H&E x 10

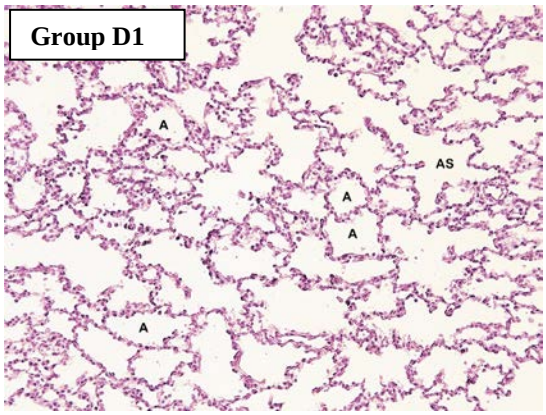

Fig. 4: Photomicrograph of fetal rat lung of Group D1 showing large diameter of alveoli (A). H&E x 10

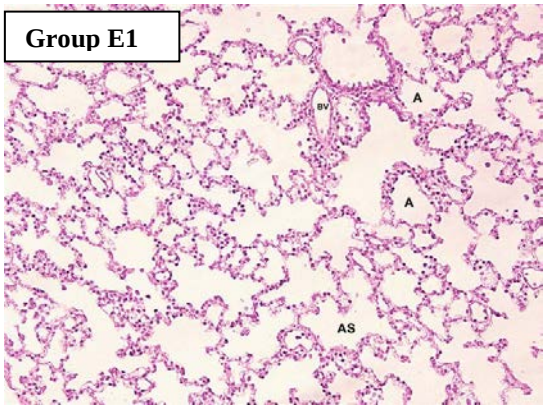

Fig. 5: Photomicrograph of fetal rat lung of Group E1 showing focal enlargement of alveoli (A). H&E x 10

0.2mg/kg subcutaneously on gestational day 16, 17 and 18 which resulted in decrease in lung's gas exchange surface area and number of alveoli in pups[16].

The increase in diameter of alveoli may be due to inhibition of alveolarization. Steroid therapy leads to increased expression of vascular endothelial growth factor, which may inhibit alveolarization and lead to abnormally large and decreased number of alveoli. These changes have been proposed to have long-term consequences that may extend into adulthood[7]. Junior et al (2008) described in his study that the lungs of the neonates from DEX treated group presented well developed numerous alveoli with varied diameter and very lean interalveolar septa, indicating an increase in the maturity of these structures[3].

CONCLUSION

The results of this research work indicate that Dexamethasone has direct influence on developing rat lung alveoli especially its diameter. This effect was comparatively more pronounced in female pups than in male pups. Use of this drug in pregnancy or in women of childbearing potential requires that the anticipated benefits be weighed against the possible hazards to the mother and embryo or fetus.

REFERENCES

1. Katzung BG. Basic and clinical Pharmacology. Chapter 39, Adrenocorticosteroids & Adrenocortical antagonists; New York: Lange; 2007, p.635-41.
2. Dexamethasone- oral [internet]. 2010 [cited 2010 Mar 21]. Available from: www.medicinenet.com/dexamethasone-oral/article.htm
3. Junior V, Teixeira A, Wanderley V, Moraes E, Araujo A. Morphological analysis of neonates of rats treated with Dexamethasone in the initial phase of pregnancy. Int J Morphol 2008; 26(3):523-7.
4. Hansen DK, Laborde JB, Wall KS, Holson RR, Young JF. Pharmacokinetics consideration of Dexamethasone- induced developmental toxicity in rats. Toxicol Sci, 1999; 48:230-9.
5. Lee CH, Peng MJ, Wu CL. Dexamethasone to prevent postextubation airway obstruction in adults: a prospective, randomized, double-blind, placebo-controlled study. Critical Care 2007; 11(4):72-9.
6. Samtani MN, Pyszczynski NA, DuBois DC, Almon RR, Jusko WJ. Modeling glucocorticoid-mediated fetal lung maturation: Temporal patterns of corticosteroids in rat pregnancy. JPET 2005:10:117-26.
7. Pua JS, Stonestreet BS, Cullen A, Shahsafaei AA, Grazyna BS. Histochemical analyses of altered fetal lung development following single vs multiple courses of antenatal steroids. J Histochem Cytochem 2005; 53: 1469–79.
8. Hauser J, Artho AD, Pilloud S, Maier C, Knapman A, Feldon J. Effects of prenatal Dexamethasone treatment on postnatal physical, endocrine and social development in the common marmoset monkey. Endocrinology 2006; 148(4): 1813-22.
9. Manojlivić M, Hristić M, Kalafatić D, Plećas B, Ugresić N. The influence of dexamethasone treatment of pregnant rats on the development of chromaffin tissue in their offspring during the fetal and neonatal period. J Endocrinol Inves 1998; 21(4): 211-8.
10. Pinkerton KE, Joad JP. The mammalian respiratory system and critical windows of exposure for children's health. Environ Hlth Perspectives 2000; 108:457-62.
11. Massaro GD, Massaro D. Formation of alveoli in rats: postnatal effect of prenatal Dexamethasone. Am J Physiol Lung Cell Mol Physiol 2002; 263(1):37-41.
12. Massaro D, Massaro GD. Formation of alveoli in rats: postnatal effects of prenatal dexamethasone. Am J Physiol Lung Cell Mol Physiol 1992; 263:37- 41.
13. Bancroft JD, Gamble M. Theory and practice of histological techniques. 5th ed. London: Churchill Livingstone; 2002.p.75.
14. Roberts M, Reiss M, Monger G. Gaseous exchange Advanced Biology. Surrey: Nelson; 2000.p.167.
15. Hyde DM, Tyler NK, Putney LF, Singh P, Gundersen HJ. Total number and mean size of alveoli in mammalian lung estimated using fractionator sampling and unbiased estimates of the Euler characteristic of alveolar openings. The Anatomical Record 2004; 274:216–26.
16. Massaro D, Massaro GD. Formation of alveoli in rats: postnatal effects of prenatal dexamethasone. Am J Physiol Lung Cell Mol Physiol 1992; 263:37- 41.

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