Comparison of the Effect of Single and Repeated Courses of Corticosteroids on Fetal Lung Maturation and Brain Growth in Pregnant Rats

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Abstract

Objective: To compare the effect of single and repeated courses of corticosteroids on fetal lung maturation, birth weight, head circumference and brain growth in fetal rats.

Methods: Forty two Sprague-Dawley rats were divided into 6 groups. Mature and premature pregnant rats were given intramuscular betamethasone (0.5 mg/kg) at 16 or at 16-18 days of gestation. Controls (mature and premature rats) received equivalent volumes of sterile normal saline. Rats were delivered at 19 (preterm) and 22 (term) days. After cesarean delivery, we measured birth weight, length, head circumference, weight of whole brain, maximal cerebral anterior-posterior length and evaluated the lungs histopathologically.

Results: There were no significant differences in birth weight and whole brain weights between the premature rats receiving one dose and repeated doses of corticosteroid. Lung maturation in premature rats revealed 71.4% glandular stage in the rats receiving one dose of corticosteroid where as 42.9% glandular stage and 42.9% canalicular stage in rats receiving multiple courses.

Conclusion: Administration of repeated courses of corticosteroids did not cause any significant differences in birth and brain weights, but increased maturation in lungs in comparison with one dose.

Keywords: Corticosteroids, fetal lung maturation, brain growth.

Gebe rat fetüslerinde tek ve tekrarlanan doz kortikosteroid kullanımının akciğer matürasyonu ve beyin gelişimi üzerine etkileri

Amaç: Tek ve tekrarlanan doz kortikosteroidlerin fetal akciğer matürasyonu, doğum ağırlığı, baş çevresi ve beyin gelişimi üzerine olan etkilerinin karşılaştırılması.

Yöntem: Kırk-iki tane Sprague-Dawley cinsi rat 6 gruba ayrıldı. Gebe ratlara gebeliğin 16, 17 ve 18. günlerinde intramuskuler betametason (0.5mg/kg) ve kontrol grubuna da salin solüsyonu verildi. Ratlar 19. gün (preterm) ve 22. gün (term) olarak doğurtuldu. Sezeryanla doğumu takiben doğum ağırlıkları, boy uzunlukları, baş çevreleri, tüm beyin ağırlıkları, tüm beyin en uzun ön-arka çapı ve beyin genişliklerinin ölçümü yapıldı. Histopatolojik olarak akciğer matürasyonu değerlendirildi.

Bulgular: Preterm kontrol grubu, tek doz kortikosteroid alan grup ve tekrarlanan doz kortikosteroid alan gruplar arasında doğum kilosu, boy uzunluğu, baş çevresi ve tüm beyin ağırlığı açısından anlamlı fark bulunmadı ($p > 0.05$). Pretermlerde histopatolojik olarak tek doz kortikosteroid verilen grupta akciğer matürasyon evresi %71.4 glandüler evre, %28.6 glandüler-kanaliküler evre, tekrarlanan doz kortikosteroid alan grupta %42.9 glandüler evre, %42.9 kanaliküler evre olarak bulundu.

Sonuç: Gebe ratlarda tekrarlanan dozlarda kortikosteroid kullanımı fetal doğum ve beyin ağırlığında tek doza göre farklılık yaratmamakta ancak akciğer matürasyonunda ilerlemeye yol açmaktadır.

Anahtar Sözcükler: Kortikosteroid, fetal akciğer matürasyonu, beyin gelişimi.

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Introduction

It is known that antenatal corticosteroid usage in preterm births causes decrease in the rates of neonatal death, respiratory distress syndrome, intraventricular hemorrhage and necrotizing enterocolitis.[1] It is showed that multiple dose of antenatal corticosteroids is related with decrease in neurons' count and degeneration of neurons in hippocampus.[2] Quinlivan et al reported that there is a significant decrease in those who were given repeated dose of corticosteroid as to control group in terms of body and organ weights, therm and preterm biometric measurements (weight, femur length, brain volume, brain weight).[3]

The purpose of this study is to determine the effect of corticosteroids on brain tissue and to compare effects of single dose and repeated dose of corticosteroids on lung maturation of premature and mature rats.

Methods

40 female Sprague-Dawley rats (250-275 g) and 15 male Sprague-Dawley rats were used as subject in this work. Rats were bred. Gestation was established by formation of solid, yellow vaginal plate. Appearance of vaginal plate was deemed as the first day of gestation. Newborns were used in the work. Rats were supplied from Experimental Animal Research Center of Medical Faculty of Dicle University. Rats used in the work were not used in any experiment before. Consent of ethic board was taken before starting the work. Rats were taken care in special cages having ventilation for 25 days and enough sunlight. Each rat was put into different cage to not have any problem with given drug doses and to prevent possible infection risk which might appear during experiment. All rats in cages were fed with pellet feed including low sodium. First 42 newborns of rats were included into the work. 6 working groups were formed:

Group 1: Premature control group which were applied no treatment, pregnant rats being applied antenatal saline betamethasone (n=7).

Group 2: Premature pregnant rats which had single dose of antenatal betamethasone (n=7).

Group 3: Premature pregnant rats which had three repeated doses of betamethasone (n=7).

Group 4: Mature control group which were applied no treatment, pregnant rats being applied antenatal saline betamethasone (n=7).

Group 5: Mature pregnant rats which had single dose of antenatal betamethasone (n=7).

Group 6: Mature pregnant rats which had three repeated doses of betamethasone (n=7).

All six groups were applied preterm cesarean birth by taking into consideration that gestation period of all pregnant rats were 21 days.

Before preterm cesarean birth, 0.5 mg/kg intramuscular saline solution was applied to Group 1; 0.5 mg/kg intramuscular betamethasone (Celestone Chronodose ampule, Eczacibasi Ilac Sanayi ve Ticaret A.S., Istanbul with the license of Schering-Plough Corporation) was applied to Group2; three doses of 0.5 mg/kg intramuscular betamethasone was applied to Group 3. Single dose of intramuscular saline solution was applied to Group 4; single dose of intramuscular and 0.5 mg/kg betamethasone were applied to Group 5; three doses of intramuscular and 0.5 mg/kg betamethasone were applied to Group 6. Drug applications were done on the 16th gestational day for group being applied single dose and on 16th, 17th and 18th gestational days for groups being applied repeated doses. The operation was done in 19th gestational day for premature groups and in 21st gestational day for mature groups. Vaginal plate formation was deemed as the first day of gestation for determination of gestation days and gestational days were found by counting next days for each rat. Alive young rats of each rat and their birth weight were determined. Tissues to be examined were removed in appropriate conditions after applying 50 mg/kg ketamine – hydrochloride and 5 mg/kg xylazine as anesthesia. They were fixed within formalin and taken into histopathologic examination. Histology expert was provided not to know which tissue was from which group.

In lung maturation of rat fetuses, pseudoglandular phase should be completed at 18th gestational day, canalicular phase should be completed at 19th-20th gestational day and saccular phase should be completed at 21st gestational day. For histopathologic determination of lung maturation, glandular phase, canalicular phase and saccular phase were used in determination as growth phases and glandular-canalicular phase and canalicular-saccular phase were used in determination as intermediate phases.

While evaluating findings obtained from the work, SPSS Windows 10.0 program was used for statistical analyzes and Post Hoc Multiple

Comparison, Mann Whitney-U and Kruskal-Wallis analyzes were used for statistical analysis method. Results were evaluated in the level of $p<0.005$ as statistical significance limit.

Results

In the determination of lung maturation phases, 19% of all subjects were found as glandular phase (Figure 1), 23.8% of them were found as glandular-canalicular phase, 42.9% of them were found as canalicular phase (Figure 2), 7.1% of them were found as canalicular-saccular phase and 7.1% of them were found as saccular phase (Figure 3). 85.7% of subjects which were applied single dose of betamethasone in mature group were found as canalicular phase and 14.3% of them were found as canalicular-saccular phase; 14.3% of subjects which were applied repeated dose of betamethasone were found as glandular-canalicular phase and 85.7% of them were found as canalicular phase; 14.3% of subjects which were applied saline solution were found as glandular-canalicular phase, 14.3% of them were found as canalicular phase, 28.6% of them were found as canalicular-saccular phase and 42.9% of them were found as saccular phase. 71.4% of subjects which were applied single dose of betamethasone in premature group were found as glandular phase and 28.6% of them were found as glandular-canalicular phase; 42.9% of subjects which were applied repeated dose of betamethasone were found as glandular phase and 14.3% of them were found as glandular-canalicular phase, 42.9% of them were found as canalicular phase; 71.4% of subjects which were applied saline solution were found as glandular-canalicular phase and 28.6% of them were found as canalicular phase.

No significant difference was found between groups which took single dose of betamethasone (101.14±32.48 mg), repeated dose of betamethasone (117.28±51.21 mg) and saline solution (171.28±53.61 mg) in terms of weight of whole brain in premature rat groups ($p>0.005$). A significant difference was found between both groups which took saline solution (335.00±117.08 mg) and single (228.14±45.52) and repeated dose of betamethasone (195.42±61.65 mg) in groups of mature rats ($p<0.005$). No significant difference was found between groups having single and repeated doses of betamethasone ($p=0.36$) (Diagram 1) (Figures 4 and 5).

Birth weight, head circumference, brain length and brain width information of premature and mature rat groups were given in Table 1 for repeated dose of corticosteroid and in Table 2 for single dose of corticosteroid.

Discussion

There are potential dangers of repeated dose of antenatal corticosteroid usage.[1,4] These are: 1.Glucose tolerance disorder 2. Osteolysis 3. Adrenal suppression 4. Dysplasia 6.Myelinization anomaly. American Collage of Obstetricians and Gynecologist (ACOG) suggests that it is suitable to give betamethasone or dexamethasone to preterms

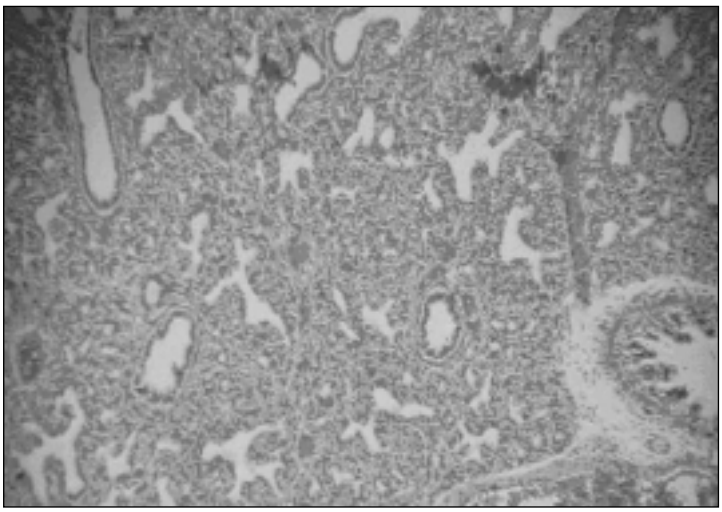

Figure 1. Glandular phase of lung maturation (H&E x 100).

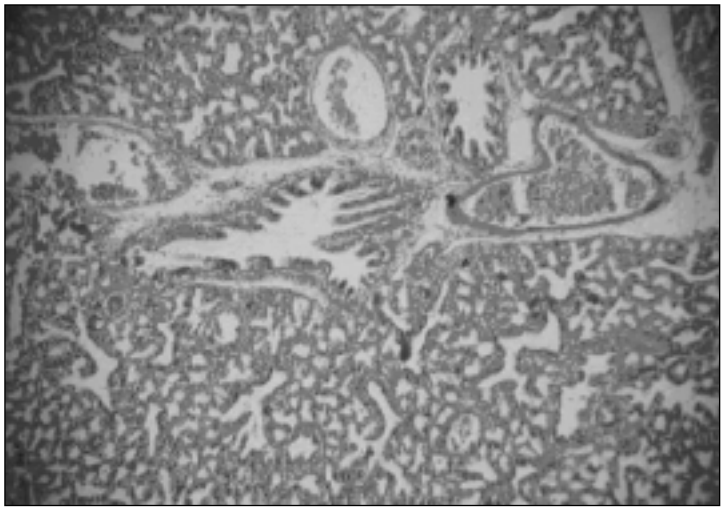

Figure 2. Canalicular phase of lung maturation (H&E x 100).

which are between 24th-34th weeks but they think that there is no enough proof for supporting repeated dose of antenatal corticosteroid.[5] We evaluated effects of antenatal single dose and repeated dose of betamethasone on dysplasia and organ maturation in our work. We determined head circumferences, lengths, birth weights, brain weights, brain widths and brain lengths of newborn subjects as the simplest developmental varieties.

Scheepens et al[6] showed that betamethasone caused somatic growth retardation and decrease in brain cell proliferation. No significant difference was found between groups which had single dose, repeated dose of betamethasone and saline solution for determining whole brain weight in premature rat groups ($p > 0.05$). A significant difference was found between both groups which took saline solution and single and repeated dose of betamethasone in groups of mature rats ($p<0.005$). The reason for not observing any significant difference in brain development of preterm rats may be that the time is not enough between corticosteroid usage and birth. Namely, even though corticosteroid usage has an effect on brain development, this effect may not be observed. Likewise, corticosteroid usage in mature rats and finding retardation within head confirm this opinion.

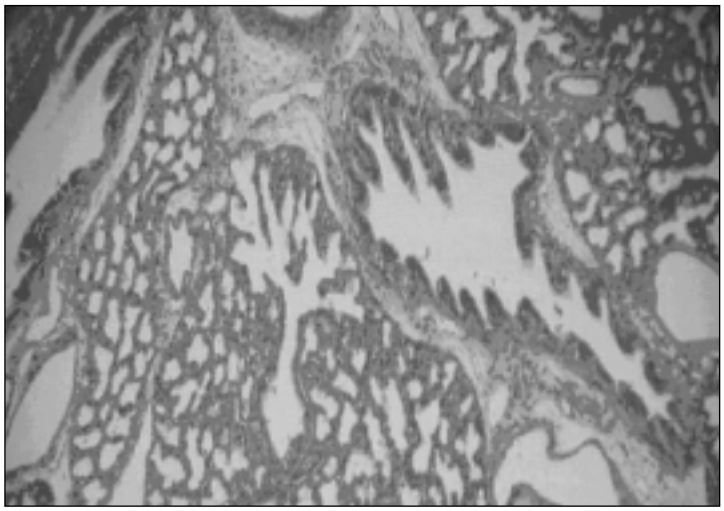

Figure 3. Saccular phase of lung maturation (H&E x 100).

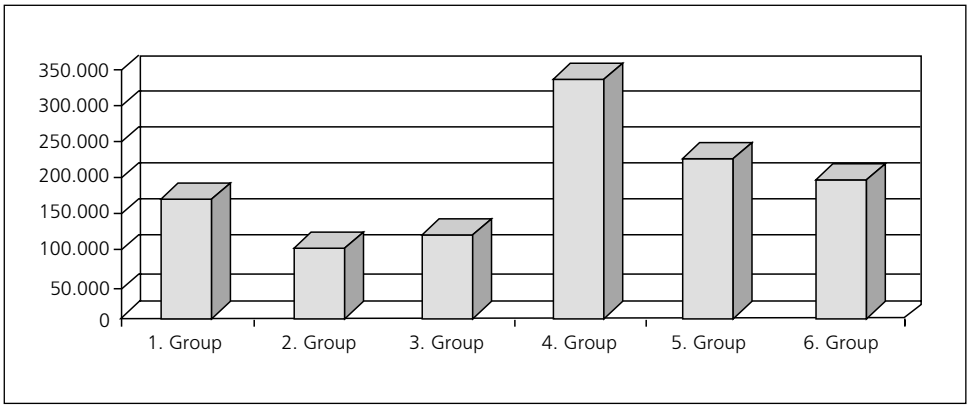

Diagram 1. Distribution of subjects as to their brain weight (mg).

Sloboda et al[7] mentioned that betamethasone application at pregnant sheeps caused a significant decrease in birth weight. It is not known exactly how glucocorticoids affect fetal growth. French et al4 showed that decrease rate in birth weights are related with dose repeat of glucocorticoid. No significant difference was found

No significant difference was found between groups which had single dose, repeated dose of betamethasone and saline solution for determining birth weight in groups of premature rats (p> 0.05). A significant difference was found between both groups which took saline solution and single and repeated dose of betamethasone for determining birth weight in groups of mature rats (p<0.005).

Noel et al[8] included pregnants into their work which took repeated dose of antenatal corticosteroid under 33 week in Western Australia and they examined effects of repeated dose of antenatal corticosteroid on birth weight and head circumference. They found that there was decrease in birth weight and head circumference in preterm pregnants which used repeated dose of antenatal corticosteroid.[8]

Table 1. Repeated dose of corticosteroid data in premature and mature rat groups.

	Premature			Mature		
Measurements	**Control (n=7)**	**Repeated dose (n=7)**	**P**	**Control (n=7)**	**Repeated dose (n=7)**	**P**
Birth weight (g)	2.62±0.22	1.97±1.42	0.57	5.48±1.33	3,40±1.11	0.001
Head circumference (cm)	2.82±0.33	2.41±1.08	0.19	3.51±0.32	3,22±0.63	0.03
Length (cm)	2.84±0.26	2.47±1.16	0.26	3.95±0.37	3,14±0.60	0.01
Brain weight (mg)	171.28±53.61	117.28±51.21	0.13	335.0±117.0	195,42±61.65	0.001

Table 2. Single dose of corticosteroid data in premature and mature rat groups.

	Premature			Mature		
Measurements	**Control (n=7)**	**Single dose (n=7)**	**P**	**Control (n=7)**	**Single dose (n=7)**	**P**
Birth weight (g)	2.62±0.22	1.85±0.25	0.19	5.48±1.33	4.28±1.36	0.04
Head circumference (cm)	2.82±0.33	2.13±0.22	0.11	3.19±0.32	3.70±0.47	0.49
Length (cm)	2.84±0.26	2.28±0.22	0.09	3.95±0.37	3.74±0.49	0.51
Brain weight (mg)	171.28±53.61	101.14±32.48	0.05	335,0±117.08	228.14±45.52	0.005

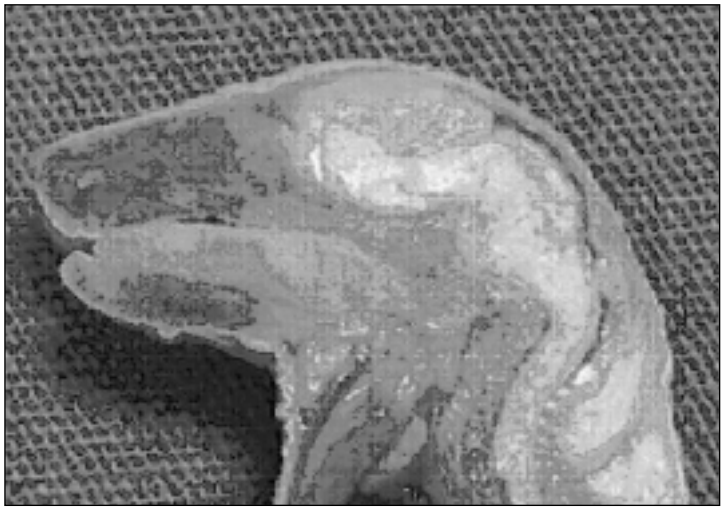

Figure 4. The head of the mature rat fetus.

In the determination of lung maturation phases, 85.7% of subjects which were applied single dose of betamethasone in mature group were found as canalicular phase and 14.3% of them were found as canalicular-saccular phase; 14.3% of subjects which were applied repeated dose of betamethasone were found as glandular-canalicular phase and 85.7% of them were found as canalicular phase; 14.3% of subjects which were applied saline solution were found as glandular-canalicular phase, 14.3% of them were found as canalicular phase, 28.6% of them were found as canalicular-saccular phase and 42.9% of them were found as saccular phase. 71.4% of subjects which were applied single dose of betamethasone in premature group were found as glandular phase and 28.6% of them were found as glandular-canalicular phase; 42.9% of subjects which were applied repeated dose of betamethasone were found as glandular phase and 14.3% of them were found as glandular-canalicular phase, 42.9% of them were found as canalicular phase; 71.4% of subjects which were applied saline solution were found as glandular-canalicular phase and 28.6% of them

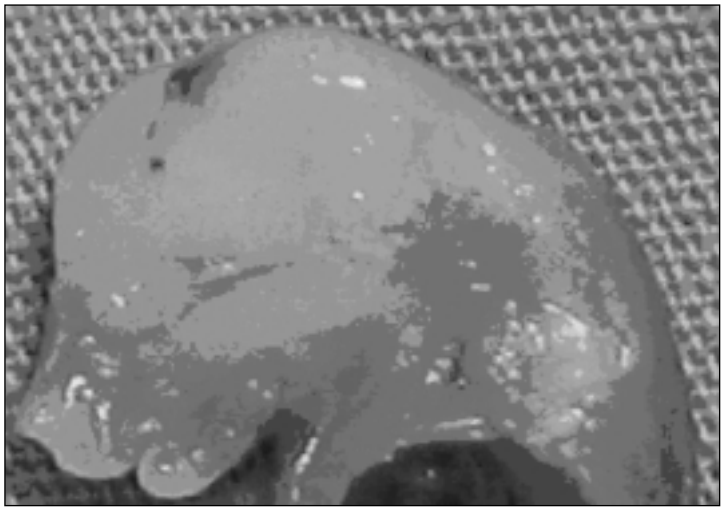

Figure 5. The head of the premature rat fetus.

were found as canalicular phase. Lung maturation phase of subjects which took single dose of betamethasone in premature group was found lower than group which took repeated doses of betamethasone. Also it is important that lung development in control group of preterm rats is higher than group which was applied single dose of corticosteroid.

Stephan et al compared the effects of single and repeated doses of antenatal betamethasone in between 24th-34th gestational weeks on neonatal sepsis and death. They reported that they got similar results in both groups in terms of respiratory distress syndrome and grade 3,4 intraventricular hemorrhage incidence risk.[9]

Consequently, we observed that repeated dose of betamethasone increased lung maturation in rats when compared with single dose. We found that there is no difference between effects of single and repeated doses of corticosteroid on brain and birth weight in premature rats. But the reason of this may be that the time is not enough between corticosteroid usage and birth.

References

1. NIH Consensus Development Panel on the Effect of Corticosteroids for Fetal Maturation on Perinatal Outcomes. Effect of corticosteroids for fetal maturation on perinatal outcomes. *J Am Med Assoc* 1995; 273: 413-18.
2. Uno H, Lohmiller L, Thieme C, Kemnitx JW, Engle MJ, Rocker EB et al. Brain damage induced by prenatal exposure to dexamethasone in fetal rhesus macaques. I: hippocampus. *Dev Brain Res* 1990; 53: 157-67.
3. Quinlivan JA, Archer MA, Dunlop SA, Evans SF, Beazley LD, Newnham JP. Fetal growth retardation, particularly within lymphoid organs, following repeated maternal injections of betamethasone in sheep. *J Obstet Gynecol Res* 1998; 24: 173-82.
4. French NP, Evans SF, Godfrey KF, Newnham JP. Repeated antenatal corticosteroids: size at birth and subsequent development. *Am J Obstet Gynecol* 1999; 180: 114-121.
5. Bennet L, Kozuma S, McGarrigle HH, Hanson MA. Temporal changes in fetal and cardiovascular, behavioural, metabolic and endocrine responses to maternally administered dexamethasone in the late gestation sheep. *Br J Obstet Gynaecol* 1999; 106: 331-339.
6. Scheepens A, van de Waarenburg M, van den Hove D, Blanco CE. A single course of prenatal betamethasone in the rat alters postnatal brain cell proliferation but not apoptosis. *J Physiol* 2003; 552: 163-75.
7. Sloboda DM, Newnham JP, Challis JRG. Effects of repeated maternal betamethasone administration on growth and hypothalamic-pituitary-adrenal function of the ovine fetus at term. *J Endocrinol* 2000; 165: 79-91.
8. Noel P, French MB, Ronald Hagan MB. Repeated antenatal corticosteroids: Size at birth and subsequent development. *Am J Obstet Gynecol* 1999; 180: 114-21.
9. Stephen T, Vermillion MD, David E, Soper MD, Roger B, Newman MD. Neonatal sepsis and death after multiple courses of antenatal betamethasone therapy. *Am J Obstet Gynecol* 2000; 183: 810-4.