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Effects of maternal dexamethasone exposure on hematological indices in the male offspring

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ABSTRACT

Maternal treatment with dexamethasone in threatening preterm delivery leads to high basal corticosterone level in the offspring. Excess glucocorticoids may inhibit the production of interleukin. This study examined the effects of prenatal and lactational dexamethasone exposure on hematological parameter in male offspring. The rats were divided into 9 groups. Group1 was administered 0.02 ml/100gbw/day normal saline throughout pregnancy. Group 2, 3, 4 and 5 were administered 100 µg/kgbw/day dexamethasone through gestation day (GD) 1-7, 8-14, 15-21 and 1-21 respectively. Group 6 was administered 0.02 ml/100gbw/day normal saline at Lactational day (LD) 1-21. Group 7, 8 and 9 were administered 100 µg/kgbw/day dexamethasone at LD 1-7, 1-14 and 1-21 respectively. The male offspring were sacrificed at 12 weeks of age for the evaluation of hematological indices. Results show that dexamethasone exposure at GD 1-7, 8-14 and 1-21 significantly ($P<0.05$) reduced PCV, hemoglobin concentration, RBC, platelet and neutrophil differential counts, raised eosinophil differential count relative to control. Exposure to dexamethasone at LD 1-14 and 1-21 significantly ($P<0.05$) reduced RBC and platelet counts but it raised MCV and MCH relative to control. This study suggests that prenatal and lactational dexamethasone administration may affect the hematological indices in the male offspring.

Keywords: Dexamethasone, prenatal, lactational, hematological indices, fetal, corticosterone.

INTRODUCTION

Fetal exposure to stress and its glucocorticoids hormone mediators exerts influences on organ growth, development and subsequent offspring physiology (Drake et al., 2007). In clinical situations, sources of maternal exposure to glucocorticoids includes; maternal stress, treatment with synthetic glucocorticoids in threatening preterm delivery, treatment of medical condition such as asthma (Singh et al., 2012). Pregnant women who are at risk of delivering a child with congenital adrenal hyperplasia are also likely to receive doses of dexamethasone (a

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synthetic glucocorticoid that freely crosses the placenta) that are 60-fold higher than mid-gestation glucocorticoids values (Peiser et al.,2010), to reduce genital virilisation of the female fetus (Clayton and Brock, 2012).

Epidemiological studies in human and experimental studies in animal model have shown that individual tissues and whole organ systems can be programmed in utero during critical periods of development with adverse consequences on their function in later life (Fowden and Forhead, 2004). For instance, androgen dependent programming effects are usually targeted at the male programming window between gestation days 15.5-19.5 in rats (Drake et al., 2009). Recent reports have shown that exposure to maternal stress during lactation can adversely affect the maternal hypothalamic pituitary adrenal function (Tilbrook et al., 2006). This may result in altered corticosterone level in the offspring.

It has been reported in human and animal studies that early exposure to glucocorticoid could also retard growth and subsequent development of hypertension, insulin resistance, type 2 diabetes and cardiovascular disease (Drake et al., 2007). Moreover, Llorente et al. (2002) have reported that prenatal stress may decrease some immune parameters in the blood. Normally, excess glucocorticoids may inhibit the production of GM-CSF, IL-6, IL1 and TNFα (Pazirandeh et al., 2002). The effects of prenatal and lactational exposure to synthetic glucocorticoids on hematological indices have not been well reported in literature. Therefore, this study aims at evaluating the effects of prenatal and lactational dexamethasone (synthetic glucocorticoids) administration on hematological indices in Wistar rats.

MATERIALS AND METHODS

Animal treatment

Forty-five (45) female albino rats (150-180 g) were purchased from central animal house of University of Ibadan. After two weeks of acclimatization, animals in proestrous were exposed to matured male overnight and the presence of sperm in their vaginal in the next morning mark gestation day 1 (GD1). All animal experiments were conducted in accordance with ethical norms on Animal Care and Use approved by IMRAT and Faculty of Basic Medical Sciences, College of Medicine, University of Ibadan. After pregnancy has been established, animals were randomly divided into nine groups (n=5) and treated accordingly (Table 1). Administration was between 09.00 am and 10.00 am daily. Dexamethasone (100 µg/kg bw/day) was administered to the treated groups and 0.02 ml/100g bw/day normal saline was administered in the control. All administrations were done subcutaneously. The male offspring were allowed to grow to adulthood (12 weeks of age). Number of male offspring collected is shown in Table 1.

This study was carried out at the Department of Physiology, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Blood count

Blood sample were obtained at 12 weeks of age from ocular sinus into heparinize tube for the determination of blood parameters. PCV was measured by the microhaematocrit technique using Hawsksley microhaematocrit centrifuged and spinning for 5 min at 12,000xg before reading with hematocrit reader. Hemoglobin levels were measured by the cyanomethaemoglobin method using CE 404 colorimeter (Cecil

Instrument). The RBC, WBC and platelet counts were done by haemocytometer method. MCV, MCH and MCHC were calculated indirectly by using standard formular. Differential count was done from prepared blood smear on a clean glass slide observed under light microscope.

Statistical analysis

Data are expressed as mean ± standard error of mean (SEM) Statistical comparisons were performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test to compare the means of the different treatment groups. Differences between the treatment groups with a p- value < 0.05 were considered significant. Data were analysed with the use of Graphpad Prism Version 5.0 for Windows (GraphPad® Software, San Diego, CA, USA.

RESULTS

Effects of prenatal dexamethasone exposure on hematological indices

Male offspring born to mother treated with 100 µg/kgbw/day dexamethasone (Dex) during gestation (Gd) day 1-7, 8-14 and 1-21 shows a significant (P<0.05) reduction in PCV and RBC compared to control(Table 2). Blood hemoglobin level was also significantly reduced in the offspring born to mother treated with 100 µg/kgbw/day Dex during GD 1-7, 8-14 and 1-21 when compared to control and Dex Pn 15-21 (P<0.01) (Table 2). However, exposure to dexamethasone during GD 15-21 had no significant effects on PCV, RBC, and hemoglobin level (Table 2). MCH, MCHC and MCV were also not significantly (P>0.05) different in all the treated groups (Table 4).

Blood platelet count was significantly (P<0.05) reduced in all the treated groups when compared to control (Table 2). Total WBC count and differential count for lymphocyte were not significantly (P>0.05) different from control (Table 2 and 3). Meanwhile, differential count for neutrophil was significantly (P<0.05) reduced in the Dex Pn 1-21 group compared to control (Table 3). Differential counts for eosinophil was significantly higher in all the treated groups when compared to control (Table 3).

Effects of lactational dexamethasone exposure on hematological indices

Exposure to 100 µg/kgbw /day dexamethasone during lactation from Lactational day (LD) 1-14 and 1-21 significantly (P<0.01) reduce the RBC count when compared to the control and when compared to Dex LD 1-7 (Table 5). MCV and MCH were also significantly (P<0.01) higher in the Dex LD 1-14 and Dex LD1-21 group when compared to control and Dex LD1-7 groups (Table 7). MCHC level was however not significantly different in all the treated groups (Table 7). Blood platelet count was also significantly reduced in the Dex LD 1-7 (P<0.05) and Dex LD1-14 (P<0.01) groups when compared to control and when compared to Dex LD 1-21 groups (Table 5). Moreover, total WBC count and differential count are not significantly (P>0.05) different in all the treated groups (Table 6).

Table 1: Treatment of animals and number of offspring collected.

Group	Treatment	No of male Offspring included
Control Pn	0.02ml/100g bwt/day Normal saline (GD 1-21)	7
Dex Pn 1-7	100µg/kg bwt/day Dexamethasone (GD 1-7)	7
Dex Pn 8-14	100µg/kg bwt/day Dexamethasone (GD 8-14)	7
Dex Pn 15-21	100µg/kg bwt/day Dexamethasone (GD 15-21)	7
Dex Pn 1-21	100µg/kg bwt/day Dexamethasone (GD 1-21)	7
Control LD	0.02ml/100g bwt/day Normal saline (PND 1-21)	6
Dex LD 1-7	100µg/kg bwt/day Dexamethasone (PND 1-7)	6
Dex LD 1-14	100µg/kg bwt/day Dexamethasone (PND 1-14)	6
Dex LD 1-21	100µg/kg bwt/day Dexamethasone (PND 1-21)	6

Dex (Dexamethasone), LD (Lactational), Pn (Prenatal)

Table 2: Effects of prenatal dexamethasone treatment on whole blood count and heamoglobin concentration.

Treatments	Hematological Indices				
	PCV (%)	RBC (10^6/ml)	Heamoglobin (g/dl)	WBC (10^3/ml)	Platelet count (10^3/ml)
Control Pn	48 ±0.856	8.176±0.24	16.57±0.088	5937.5±288.95	94750±672.5
Dex Pn 1-7	42.33±1.453*	6.98±0.29*	13.83±0.731**#	5450±407.23	81333±982***###
Dex Pn 8-14	42.66±1.2*	7.17±0.13*	13.23±0.541**##	5362.5±134.44	73666±296***###
Dex Pn 15-21	46±0.5774	7.67±0.13	16.00±0.057	5066.66±44.096	57333±881***###
Dex Pn 1-21	43±0.8165*	7.065±0.16*	13.36±0.338**##	5532±54.23	26457±264***###

*p= 0.05,** p=0.01, ***p<=.001; # p= 0.05, ## p=0.01, ### p=0.001, N=7

*, shows significant different between the group and the control; #, shows significant different between the group and Dex Pn 1-7. Dex (Dexamethasone), Pn (Prenatal).

Table 3: Effects of prenatal dexamethasone treatment on differential count.

Treatments	Hematological indices			
	% Lumphocyte	% Neutrophil	% monocyte	% eosinophil
Control Pn	70.6±2.943	25.8±1.456	2.4±0.4	1.2±0.3
Dex Pn 1-7	73.0±0.5774	21.33±1.453	3.30.67	2.33±0.1
Dex Pn 8-14	72.0±0.632	21.42±1.21	2.9±0.27	2.4±0.15*
Dex Pn 15-21	71.33±0.8819	23.3±1.453	2.33±0.05	2.66±0.3*
Dex Pn 1-21	77.0±3.488	18.00±3.136*	2.0±0.408	3.0±0.408**

*p= 0.05,** p=0.01, n=7,

*, shows significant different between the group and the control. Dex (Dexamethasone), Pn (Prenatal).

Table 4: Effects of prenatal dexamethasone treatment on RBC indices.

Treatments	Hematological indices		
	MCV (fl)	MCH (ρg)	MCHC g/dl
Control Pn	61.024±0.89	20.39±0.6079	33.434±1.018
Dex Pn 1-7	60.687±0.464	19.8±0.222	32.637±0.5999
Dex Pn 8-14	61.34±0.5023	20.013±0.1087	32.54±0.7151
Dex Pn 15-21	60.81±0.6847	20.276±0.3021	32.503±0.8218
Dex Pn 1-21	61.91±0.362	19.462±0.3736	32.0075±0.4962

n=7, Dex (Dexamethasone), Pn (Prenatal).

Table 5: Effects of lactational dexamethasone treatment on whole blood count and heamoglobin concentration.

Treatments	Hematological indices				
	PCV (%)	RBC (10^6/ml)	Heamoglobin (g/dl)	WBC 10^3/ml)	Platelet count 10^3/ml)
Control LD	35.84±1.249	5.16±0.1704	11.00±0.6245	5.846±0.747	29766±983.8
Dex LD 1-7	33.19±1.946	4.94±0.01	10.13±0.7446	6.12±0.6879	19666±128.7**#
Dex LD 1-14	35.117±0.8967	4.45±0.041**#	11.097±0.0664	6.71±0.6505	14766±956.3**##
Dex LD 1-21	34.69±0.2155	4.37±0.1396**#	11.40±0.802	6.307±0.8570	12866±126.6**##

NB: *p= 0.05,**p=0.01,; # p= 0.05, ## p= 0.01 , n=6;

*, shows significant different between the group and the control; #, shows significant different between the group and Dex LD 1-7. Dex (Dexamethasone), LD (Lactational).

Table 6: Effects of lactational dexamethasone treatment on differential count.

Treatments	Hematological indices			
	% Lumphocyte	% Neutrophil	% monocyte	% eosinophil
Control LD	72±4.894	22.9±0.23	2.4±0.4	2.2±0.3
Dex LD 1-7	73.37±0.797	21.21±0.34	3.30.67	2.33±0.1
Dex LD 1-14	71.683±0.858	23.63±0.28	2.9±0.27	2.4±0.15
Dex LD 1-21	73.66±2.404	22.21±0.32	2.33±0.05	2.66±0.3

n = 6. Dex (Dexamethasone), LD (Lactational).

Table 7: Effects of lactational dexamethasone treatment on RBC indices.

Treatments	Hematological indices		
	MCV (fl)	MCH (ρg)	MCHC (g/dl)
Control LD	69.330±0.8819	21.35±0.3926	30.08±0.5696
Dex LD 1-7	67.00±2.309	20.466±0.5175	30.467±0.5239
Dex LD 1-14	82.00±0.5774**##	24.676±0.2233**##	30.06±0.1002
Dex LD 1-21	80.00±2.646*##	24.733±0.8293**##	30.867±0.5548

*p= 0.05,**p= 0.01, #p = 0.05, ##p=0.01, N=6.

*, shows significant different between the group and the control; #, shows significant different between the group Dex LD 1-7. Dex (Dexamethasone), LD (Lactational).

DISCUSSION

The present study examined the effect of prenatal and lactational dexamethasone (synthetic glucocorticoids) exposure on hematological indices in male offspring.

Prenatal dexamethasone exposure during early, mid or throughout gestation was found to reduce PCV, hemoglobin concentration and RBC count. PCV is the variable normally used to assess the basic status of the erythron; therefore the reduced PCV may be due to reduction in the RBC production. Erythropoietin hormone stimulates the production of RBC and synthesis of hemoglobin (Ganong, 2005). Prenatal excess cortisol inhibits erythropoietin production in fetal sheep (Lim et al., 1996). Dexamethasone treatment of pregnant ewes in mid-term gestation decreased fetal but not adult renal erythropoietin messenger RNA (mRNA) levels (Lim et al.,1996). Thus, there is evidence that glucocorticoids may have a negative regulatory effect on erythropoietin gene expression. This may be responsible for the reduction in RBC count and hemoglobin concentration observed in this study. However, Red blood cell indices (RBC Indices) such as MCV, MCH, MCHC were not significantly affected by the prenatal dexamethasone exposure.

This study also indicates that prenatal exposure to dexamethasone in the early, mid or late gestation also leads to reduce platelet count. The reason for this is not known but it has been previously shown that plasma cortisol level is increased in offspring by the prenatal stress exposure (Kapoor et al.,2006) and excess cortisol is a potent inhibitor of cytokines such as IL 1 and 6 (Pazirandeg et al., 2002). These two cytokines stimulate erythrocytes, granulocyte, megakaryocyte cell line (Ganong, 2005). Therefore, the reduced

platelet count may be secondary to the inhibition of IL 1 and 6.

Although, Total WBC count and differential count for lymphocyte were not significantly affected by the treatment but differential count for neutrophil was reduced in the group that was exposed to dexamethasone throughout the gestation period. In addition, differential count for eosinophil was significantly increased by the treatment. This is contrary to reduced eosinophilia following administration of glucocorticoids in asthmatic patient (Newton, 2000). The reason for this is not known, but it may be an adaptive response to stressful perinatal life. However, Newton also reported from Reichardt and Schutz, that glucocorticoids increase eosinophil proliferation and its mechanism is not well established (Newton, 2000). Similar increased in eosinophil differential count have also been reported due to maternal stress during late gestation in rats (Llorente et al., 2002).

Another important finding of this study is that administration of dexamethasone in the dams during lactation reduced the RBC count and also raises RBC indices especially MCV and MCH in the male offspring. It has earlier been observed that MCH value normally parallel the MCV value and defect in nuclear maturation as seen in megaloblastic anemia results in large oval erythrocytes with a normal hemoglobin content, the MCV and MCH are increased while the MCHC remain normal (Aslinia et al., 2006). It is possible that lactational dexamethasone exposure also induces this kind of defect in nuclear maturation.

Reduced platelet cell count found in this study due to lactational exposure, might also be as a result of the inhibitory effect of excess glucocorticoids on the production of platelet cell. However, dexamethasone exposure during lactation does not affect WBC count and differential count in this study.

Conclusion

This study suggests that prenatal and lactational dexamethasone administration at certain critical period of development affect hematological parameter (particularly RBC indices and platelet count) in the male offspring of exposed mother.

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