

Antenatal Glucocorticoids & Renal Development in Rat

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ABSTRACT

Antenatal glucocorticoids are routinely given to women at risk of premature delivery before 34 weeks of gestation, to decrease the incidence and severity of respiratory distress syndrome. These hormones change the intrauterine environment and program the developing fetus in a different way.

Materials and methods: 72 pregnant albino rats were divided into control group A and experimental group B, comprising of 36 pregnant rats in each group. Animals in control group were given intraperitoneal injections of normal saline and their experimental counterparts were injected with dexamethasone, for two consecutive days on different gestational days (day 11-14 till 21-22 day). Effects in fetal kidneys were observed after birth at the age of 12 weeks.

Results; Significant reduction in renal corpuscles was noted in fetuses exposed to antenatal dexamethasone. Maximum reduction (26.79%) was noted in fetuses from mothers who received dexamethasone on 15th & 16th day of gestation.

Key words: Antenatal, renal development

INTRODUCTION

Normal fetal development depends upon the normal genetic make but intrauterine environment also has major programming influence upon the developing fetus. In some cases environmental factors may become even more important and alters the expression of fetal genome, programming the individual in a different way (Wu et al, 2004). Along with environmental factors many drugs taken by the mother during pregnancy may also affect the development of different organ systems in the fetus (Asthon, 2000). The ultimate effect depends upon the dosage and time of gestation, when drug was given.

Hormones play an important role in the development of fetus. They are derived from fetal, maternal and placental sources. The most important of these hormones is corticosteroids. Blood levels of fetal cortisol are regulated by the enzyme 11 β hydroxysteroid dehydrogenase-2 (11 β HSD-2) in placenta that converts active cortisol to inert cortisone. Deficiency of this enzyme can raise the level of cortisol above normal in different fetal organs and can down regulate growth critical genes, resulting in growth retardation.

Antenatal glucocorticoids are routinely given to women at risk of preterm delivery (Ortiz et al, 2001) before 34 weeks of gestation, to reduce the risk of respiratory distress syndrome. Exogenous steroids are not metabolized by the placental enzymes 11 β HSD-2 and are passed to fetal circulation, raising fetal glucocorticoid level above normal at time, when it is not required. The cellular and molecular changes

produced in individual tissues combine to produce integrated changes in function at the systems level (Dodic et al, 2002). Many adulthood diseases are programmed in utero by raised glucocorticoids including hypertension and diabetes (Forhead et al, 2004). One of the reasons for hypertension may be glucocorticoid induced inhibition of nephrogenesis, resulting in (Singh et al, 2007) reduced nephron number in fetal kidney (Celsi et al, 1998).

During a normal pregnancy the levels of steroids in fetal plasma is at the highest of gestation during early pregnancy and helps in placental development. It is mainly derived from the mother through a transplacental route. At this time the fetal growth is comparatively slow. At mid gestation (McNeil, 2007) fetus starts developing rapidly. The steroids levels at this time of gestation are decreased in fetal blood. Near term the fetal steroids are raised again and fetal growth rate is slowed down (Fowden et al, 1996).

MATERIALS AND METHODS

Pregnant rats were divided into two groups A (control) and B (Experimental) comprising of thirty six rats in each group. The animals in both groups were divided into six sub groups (A1 to A6 and B1 to B6), comprising of six pregnant rats in each control sub group. The animals in control group were given intraperitoneal injection of sterilized normal saline (0.1ml/100gm of body wt). The animals in experimental group were given intra-peritoneal injection of dexamethasone in a dose of 0.4mg/kg (0.04mg/100gm) of body wt, daily for two consecutive days, on different gestational days (Table 1). All animals were allowed to deliver. Total of 60 pups

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from experimental and 60 from control group were included in the study. The pups from both control and experimental groups were allowed to feed on their mothers till the age of 12 weeks when they were sacrificed. Glomerular number in left kidney was determined by alcian blue method.

Statistical analysis: One way ANOVA was applied to compare the results within the group. For comparison between control and experimental group students t test was applied. P value <0.05 was considered significant

Determination of glomerular number by alcian blue method: Glomerular number was counted in 12 weeks rats by the method used by (Ortiz et al, 2001), with some modification. Animals were weighed and anesthetized by giving intra muscular injection of katamine, 2.5 mg/100 gm of body wt (Koolhaas, 1999), followed by ether inhalation. Branula No. 24 was introduced in the abdominal aorta with well above the origin of renal artery. Alcian blue (5%) solution was injected in the aorta in a dose of 0.2 ml /100 gm of body wt, for one minute. A second bolus was given after five minutes. Five minutes after the second injection the kidneys were removed, and weighed. Each kidney was sliced in half and was incubated separately at room temperature in five ml of 27% ammonia for two hours, then in 5ml of 8N Hcl at 50°C for one hour. The suspension was diluted to a volume of 20ml and incubated overnight at 4°C. The glomeruli were counted in 20µl of suspension under light microscope. The observed number was multiplied with 1000 to get total number of glomeruli in 20 cc of suspension that indicated the total number of glomeruli in one kidney (Ortiz et al, 2003).

RESULTS

Body weight, Kidney weight and tissue body weight index: Bodyweight and kidney weight of pups from both control group (A1-A6) and experimental group (B1-B6) was taken, Tissue body weight index (TWI) was determined and average was calculated (table 2). G[#]. gestational. Student's t-test was applied to compare the results between control (A) and experimental (B) groups. Asterisks indicate significant increase in body weight, kidney weight and TWI in dexamethasone treated pups as compared to controls *P<0.05.

Number of renal corpuscles/ kidney (Alcian blue or acid maceration method): Renal corpuscles were counted in 20µl of renal suspension from both group A (control) and group B (experimental) under light microscope. The glomeruli after taking alcian blue were seen as bluish green globular structures of different size and shapes, (Fig.1,2,3,4), average glomerular number in both groups (Table 3)

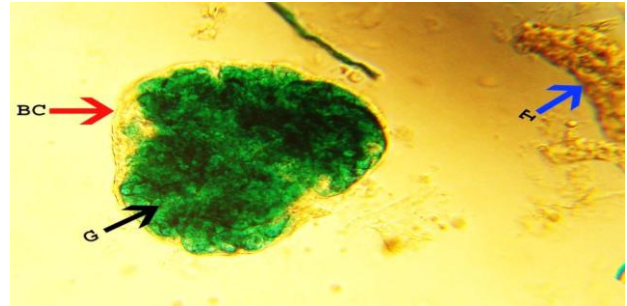


Fig 1: Microphotographs of renal smear prepared from pups 12 weeks of age from group A, showing, G, glomerulus, BC, Bowman's capsule T renal tubule. Alcian blue (in vivo) 400X

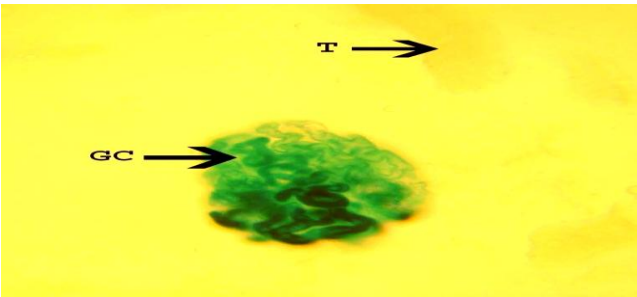


Fig. 2: Microphotograph of renal smear prepared from pups 12 weeks old pups from group A, showing GC, glomerular capillaries and T, tubules. Alcian blue (in vivo) 400X

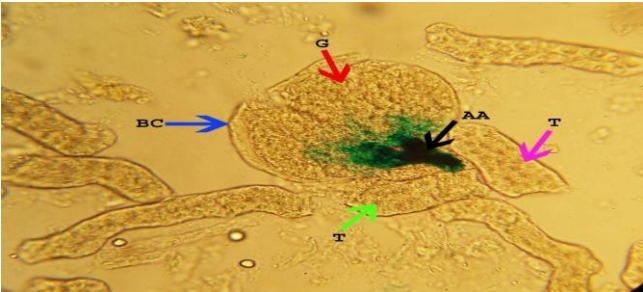


Fig. 3: Microphotograph of renal smear prepared from 12 weeks old pups from group B, showing G, glomerulus, BC, Bowman's capsule, T, renal tubule and AA, afferent arteriole. Alcian blue (in vivo) 400X

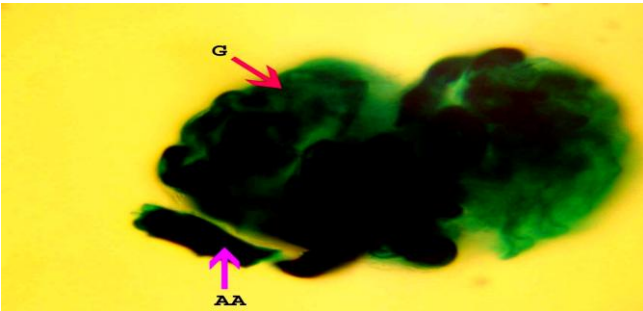


Fig. 4: Microphotograph of renal smear prepared from 12 weeks old pups from group B, showing G, glomerulus and AA, afferent arteriole. Alcian blue (in vivo) 400X

Table 1: Different Animal groups (Maternal) used during the study

Sub Group/ Treatment	Control (A) Normal saline,0.1 ml/100gm	Experimental (B) Dexamethasone 0.04mg/100gm	Gestational day for giving injections
1	A1	B1	11 th & 12 th
2	A2	B2	13 th & 14 th
3	A3	B3	15 th & 16 th
4	A4	B4	17 th & 18 th
5	A5	B5	19 th & 20 th
6	A6	B6	21 st & 22 nd

Table 2: Comparison of Body weight, kidney weight and Tissue weight index (TWI±SD) of pups from control group (A1-A6) and experimental group (B1-B6).

Parameters G [#] day of Treatment (Maternal group)	Body weight (gm ± SD)		Kidney wt (gm ± SD)		Tissue body weight (TWI)	
	A	B	A	B	A	B
11 & 12 (1)	101.2±13.97	172.4*±6.1	0.961±0.05	1.487±0.161	0.863±0.08	0.862±0.07
13 & 14 (2)	122.2±15.56	161.3*± 9.18	0.1078±0.36	1.381 ±0.22	0.882±0.06	0.854±0.13
15 & 16 (3)	138.4±20.13	159.5*±12.01	1.224±0.34	1.3679±0.264	0.93±0.083	0.867± 0.168
17 & 18 (4)	123.2±17.36	176*±11.0	1.102±0.158	1.716*± 0.16	0.89±0.076	0.975± 0.107
19 & 20 (5)	121.4±18.41	180.1*±15.65	1.042±0.176	1.5301±0.119	0.863±0.078	0.910± 0.297
21 & 22 (6)	124.8±16.36	172.1*± 13.63	1.049±0.176	1.409 ± 0.24	0.858±0.015	0.809 ±0.107

Table 3: Comparison of Renal corpuscular number±SD in left kidneys of 12 weeks old pups from control group (A1-A6) and experimental pups (B1-B6). (Alcian blue method)

G [#] . day of Treatment (Maternal group)	R [#] . corpuscular number±SD A	R-corporcular number±SD B	%age reduction in R. corpuscles in group B
11 & 12(1)	33200±1861	32600±2010	1.80%
13 & 14(2)	34400±1496.66	29400±1600**	14.53%
15 & 16(3)	33600±2653.3	24600±2374.9***	26.79%
17 & 18(4)	34800±1600	26400±1959***	24.14%
19 & 20(5)	32400±1658.31	29200±1280.6**	9.88%
21 & 22	32600±1788.84	31200±1612.5	4.29%

G[#]. day, gestational day, R[#]. Renal, Student's t-test was applied to compare the glomerular number between control (A) and experimental (B) groups. **P <0.01, ***P <0.001

DISCUSSION

The development of embryonic kidney is controlled by many regulators and transcription factors (Bouchard et al, 2004), which are influenced by the level of hormones (specially the cortisol), in fetal plasma (Fowden, 1995; Wood et al, 2009). Singh (2007) and Ortiz (2001) had shown association between prenatal corticosteroids administration and development of hypertension in adult life, due to reduced number of nephron in the kidneys.

In current study, body weights of pups from all experimental groups were significantly higher at the age of 12 weeks (P <0.001) (table 2). The results are in agreement with Ortiz (2001), who also observed that post natal body weights at the age of 60 days were higher in pups treated with dexamethasone, than controls. In another research by Dagan (2006), dexamethasone treated fetuses had lower birth weight than control littermates that was normalized at the age of 8.0 weeks. They did not follow the pups

further.

The development of postnatal obesity as was observed in present study may be due to inhibition of lipolysis, resulting in deposition of fat in the body. So individuals, who had received dexamethason in prenatal period, are heavier than control when born and obese as adults (Gatford, 2000).

In adult experimental rats, of 12 weeks of age, kidney weights were significantly (P<0.05) higher in all groups (except B3) (Table 2), than control rats of the same age but tissue body weight index (TWI) was not different significantly. These observations are with confirmation to the findings of Ortiz (2001) and Dickinson (2007). A normal kidney weight, in spite of reduced number of nephrons in present study may be due to compensatory hypertrophy of glomeruli and renal tubular dilatation as described by Dickinson (2007) and Moritz (2002).

Nephron number: In 12 weeks pups from experimental groups, significant (P<0.05) reduction in Renal corpuscles was found in Group B3 and B4

(Table, 3). In this group 26.79% reduction in renal corpuscular number was found in experimental group (B3) followed by, B4 (24.14%) and B2 (14.53%) (Table. 3, fig. 5). The minimum reduction in renal corpuscles was found in B1 (1.8%) (Table 3, Fig 5). The highly significant ($P < 0.001$) difference from control was noted in groups B2 to B4. In B1, B5 and B6 the reduction was not significant. Results are in accordance with Ortiz and colleagues (2001 and 2003) and Singh (2007) who noted 30%, 20% and 19-21% reduction respectively in glomerular number in rats treated with dexamethasone on day 14 and 15 of gestation. The maximum reduction in these studies was noted, when rats were treated on 15th and 16th day of gestation. Moritz and colleagues (2009) have reported 20-40 reduction in nephron number with short prenatal dexamethasone treatment in different animal models.

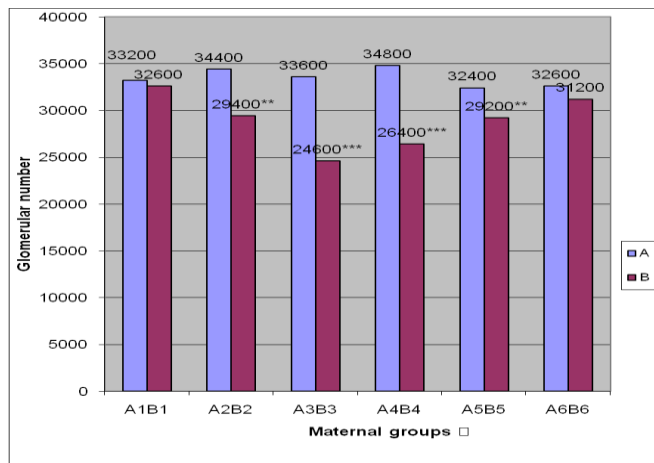


Fig. 5: Histogram showing the effect of dexamethasone on the glomerular number in left kidney, of 12 weeks old pups, from control group (A1-A6) and experimental group (B1-B6). (Alcian blue method). ** $P < 0.01$, *** $P < 0.001$

Many other researchers (Marchand et al, 2001; Seckl, 2001; Hoppe et al, 2007 and Dickinson et al, 2007) have also observed nephron reduction in kidneys of dexamethasone treated pups. The maximum effect of prenatal dexamethasone was observed, when developing kidney was in pre-glomerular stage, which corresponds to 13 to 18 days of gestation (Woods and Rasch 1998), in rats.

CONCLUSION

Prenatal dexamethasone treatment of mothers results into reduced nephron number in the fetuses in post natal period and adulthood. It is therefore suggested that steroids should be given with caution to pregnant mothers and dose should be properly calculated.

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