CYCLOSPORIN INDUCED EFFECTS ON FOETAL KIDNEY IN ALBINO MICE

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Background: Cyclosporin A (CsA) is an immunosuppressive agent which is used to prevent graft rejection and to treat autoimmune disorders. Its teratogenic effects remain unexplored despite its extensive use even during pregnancy. Current study was, therefore, undertaken to investigate the effects of CsA on the developing kidney. **Methods:** Twelve pregnant mice were divided into two groups, A and B, having six animals each. Cyclosporin was freshly prepared in normal saline daily and administered subcutaneously by a single dose of 50 mg/kg in the morning to experimental group B during pregnancy from day 0 to day 18. The control group A was given comparable volume of normal saline only. The pregnant mice were sacrificed at the end of experimental period. The foetal kidneys were dissected and fixed in 10% formalin for histological preparations. **Results:** The results showed that weight of the foetuses and their kidneys exposed to CsA was consistently reduced. The mean weight of the foetuses exposed to CsA was 1.34±0.08 g as compared to 1.48±0.18 g in the control group whereas the mean kidney weight from CsA treated group was 9.47±0.27 mg when compared to the control having 10.79±0.53 mg. Morphometric analysis revealed reduction in total number of glomeruli and hypertrophy of remaining glomeruli. The total number of glomeruli/mm^2 in the kidneys from CsA treated group was 26.85±4.43 as compared to 41.33±3.66 from the control group and the mean diameter of glomeruli from the foetuses of groups A and B was 7.11±0.47 mm and 8.66±0.63 mm respectively; the differences between the groups A and B of the animals on all the parameters above were statistically significant (p<0.000). **Conclusion:** The results of the investigation indicated that CsA administration to the pregnant dams produced deleterious effects of on the developing kidney in mice. On the analogy of the results, comparable effects of CsA are expected in case of human; this, however, needs further investigations.

Keywords: Cyclosporin A, nephrotoxicity

INTRODUCTION

The use of Cyclosporin A (CsA) has increased substantially for reason of increasing numbers of organ transplant and treatment of autoimmune diseases.[1] Renal transplant is becoming a common place and many of the recipients are pregnant mothers who are given CsA as a part of the long term treatment even during pregnancy; this has got its own risks. Pregnancy is regarded as a period of immunological tolerance because the foetus is linked to an allograft. The exposure of developing foetuses had been associated with increased incidence of spontaneous abortion, prematurity and intrauterine growth retardation among CsA treated mothers.[2] CsA and its metabolites cross the placental barrier and enter the foetal circulation, therefore, inevitably exposing it to potential foetotoxic and teratogenic effects of the drug throughout the period of development.[3,4] Previous studies showed that a prenatal exposure to CsA induced vacuolization of proximal tubular cells in rats,[5] and impaired post natal nephron differentiation in newborn rats[6]. Moreover, developmental effects have been reported after exposure of developing kidneys to CsA.[7] More recent study showed that administration of 10 mg/kg/day of CsA, from 14th to the 18th day of gestation, an early period of nephrogenesis, induced 25% nephron deficit in newborn rabbits. The pups exposed antenatally to CsA, were reported to have in sequence: a) Permanent nephron deficit, b) Glomerular tubular and intrarenal haemodynamics dysfunction, c) Enlarged kidneys with numerous tubular and glomerular lesions and d) An endothelin-dependent systemic hypertension that worsened with age.[6,8] Previous histological and mophometric approaches demonstrated that *in vitro* exposure to CsA not only induced a nephron deficit but also produced cellular lesions that were likely to further reduce the nephron endowment later in life.

In view of increasing scope of using immunosuppressant and paucity of reports on its effect on developing kidney the present study was designed to investigate the effects of CsA on kidney of foetuses when their dams were treated with CsA.

MATERIAL AND METHODS

Sixteen mice (6–8 week old) weighing 25–30 gm were used; comprising twelve females and four males. They were kept under standard condition of temperature (24±1 ºC) and humidity (55±5%) with regular 12 hour light/ dark cycle; the animals were fed with pellet food and tap water *ad libitum*. Three females and one male mouse were housed in a single cage for mating.[9] When pregnancy was confirmed by vaginal plug, twelve pregnant mice were divided into 2 groups, having 6 animals each. The experimental group B was subjected to single daily subcutaneous injections of 50 mg/kg Cyclosporin A prepared in normal saline, for 18 days. The control group received daily subcutaneous injections of comparable volume of normal saline for 18 days during gestation. The pregnant mice were sacrificed on 18th day of gestation. The foetuses were removed, examined macroscopically and weighed; their kidneys were removed, dissected

and fixed in 10% formalin for histological examination. Kidney pieces were processed in a usual way to prepare paraffin blocks; 5 µm thick sections were obtained, using rotary microtome and, those were mounted on albuminised glass slide before staining with Haematoxylin and Eosin (H&E) for study with the light microscope; Periodic acid Schiff (PAS) stain was also used for the demonstration of basement membranes. The nephrogenic zone was identified as an area containing nephrogenic mesenchyme and, was situated immediately beneath the renal capsule.[10] For morphometric analysis cortical height and nephrogenic zone thickness was measured at five different points perpendicular to the cortex from capsule to arcuate arteries, using linear micrometer and ×10 objective; the points were selected on going around the circumference of the cortex from one pole of the kidney section to the other.[10,11] The glomeruli were counted irrespective of their size and shape by fractionator method.[12] The glomeruli were identified by their tuft of capillaries and surrounding Bowman's capsule.[13] Diameter of ten renal corpuscles was measured at juxtamedullary region of the cortex in PAS stained sections. The line connecting the vascular pole to the urinary pole was taken as the vertical axis, and the widest distance at a right angle to the vertical axis was regarded as the diameter of the renal corpuscle, regardless of the size,[14] and was measured between the inner edges of the thin parietal layer of the cells forming Bowman's capsule.[15]

The data was analysed using SPSS 16.0. Mean±SD were given for normally distributed quantitative variables. Two-Independent sample *t*-test was applied to observe group mean differences. Value of $p<0.05$ was considered as statistically significant.

RESULTS

Morphologic data at birth are summarised in Table-1. The maternal CsA (50 mg/kg/day) treatment during pregnancy had deleterious effect on foetal growth, as confirmed by significant reduction in average foetal birth weight, ($p<0.001$), (Table-1).

Table-1: Comparison of morphological data at birth

Group	Control n=40	Experimental n=40	*p*
Birth weight (gm)	1.48±0.18	1.34±0.08	<0.001*
Mean kidney weight (mg)	10.79±0.536	9.47±0.273	<0.001*

*statistically significant

The foetuses exposed to CsA during gestation exhibited reduced kidney weight which was consistent with their decreased birth weight. The renal cortex was significantly thinner in foetuses where their dams were exposed to CsA treatment during pregnancy as compared with those obtained from the control group (Figure-1).

In mice, nephrogenesis is reported to start from 11–12 day of gestation indicated by an active nephrogenic zone, just beneath the capsule; this comprised of aggregates of basophilic undifferentiated mesenchymal cells which lacked proper organization and were thicker in CsA treated group than in the control (Figures-2, 3).

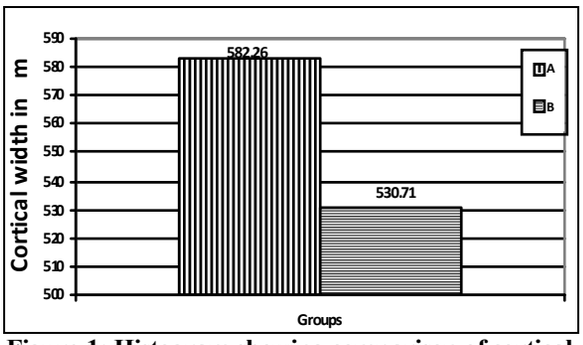

Figure-1: Histogram showing comparison of cortical width in control and the experimental groups
It is significantly thinner in the experimental than in the control group.

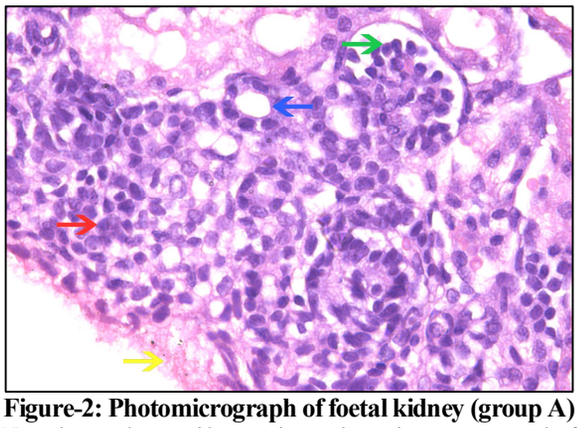

Figure-2: Photomicrograph of foetal kidney (group A)
Normal parenchyma with an active nephrogenic zone composed of nephrogenic mesenchymal cells (red arrow) subjacent to the renal capsule (yellow arrow) is seen. Different sectional profiles of tubules (blue arrow) were also seen in the renal cortex. H&E stain ×400.

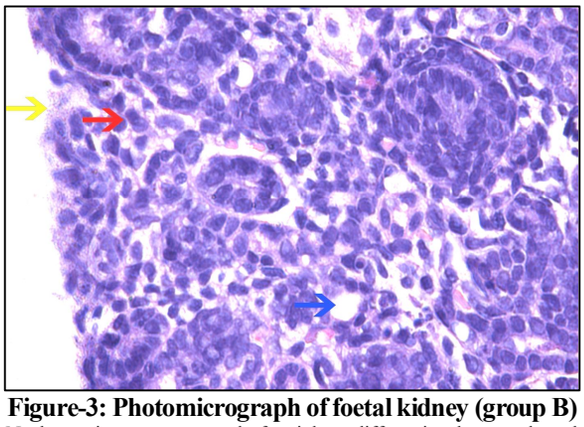

Figure-3: Photomicrograph of foetal kidney (group B)
Nephrogenic zone composed of mainly undifferentiated mesenchymal cells (red arrow) subjacent to renal capsule (yellow arrow) is seen. The renal cortex showing different profiles of tubules (blue arrow) in immature renal parenchyma. H&E stain ×400.

The morphometric analysis showed decrease in number of glomeruli/mm² in foetuses whose dams were exposed to CsA treatment, when compared to the control, the difference was statistically significant ($p<0.000$); the decrease in number of glomeruli in group B was, however, compensated by their hypertrophy where increase in size was statistically significant when compared with the control, (Table-2).

Table-2: Comparison of total number and diameter of glomeruli in experimental (50 mg/kg/day) and control groups after 18 days of gestation

Parameters of glomeruli	Group A (n=40) Mean±SD	Group B (n=40) Mean±SD	Statistical Results (*t*-test)
Total number of glomeruli/mm^2	41.33±3.66	26.85±4.43	p<0.000*
Diameter of glomeruli (mm)	7.11±0.47	8.66±0.63	p<0.000*

*statistically significant

DISCUSSION

Our investigations showed a considerable decrease in the mean body weight of the foetuses obtained from the dams receiving treatment with Cyclosporin during pregnancy when compared with those from the control group; the mean weight in the groups A and B was 1.34±0.08 g and 1.48±0.18 g respectively; when the two groups were compared the difference between the two was statistically significant. Our findings are, therefore, in accord with those reported earlier in which rabbit was used as an experimental model which revealed a statistically significant decrease in the weight of the foetuses from the dams treated with Cyclosporin when compared with those from the control.[8]

Although macroscopically foetal kidneys did not show any evidence of discernable abnormalities, the mean weight of both kidneys together was, however, reduced which was statistically significant when compared with those from the control. The morphometric analysis of glomeruli in the current investigation showed that total number of glomeruli/mm^2 from the foetal kidneys obtained from the dams of the CsA treated group was 26.85±4.43 when compared to 41.33±3.66 in the control group, the difference was statistically significant; there was an increase in size of the glomeruli of the experimental animals, presumed to indicate their compensatory hypertrophy and when compared with the control, the difference was found to be statistically significant. Similar observations were reported in rabbits treated with CsA from days 20–24 of gestation.[8] Our findings indicated thinning of cortical thickness which was reduced from 582.26±46.17 m in the control to 530.71±31.69 m in the treated group, the difference between the two was statistically significant. There was also statistically significant increase in the thickness of nephrogenic zone in treated group when compared to that in the control, the difference between the two was also statistically significant; thick nephrogenic zone in our investigations indicated delayed development of nephrons; our observations in this respect corroborate with previous findings in which it was reported that there was 25% nephron deficit in newborn rabbits; our findings of increase in thickness of nephrogenic zone was at variance with those of earlier reports of significant thinning of renal cortex after administration of CsA, 10 mg/kg per day to pregnant rabbits from 14th to 18th day of gestation.[8,16]

The results of current study also confirmed a direct relationship between growth retardation and reduction in number of glomeruli. These findings were consistent with previous studies which also reported that nephron deficit in rat, mice, rabbits, sheep and human subjects;[17] and linked it with reduced birth weight suggesting that intrauterine growth retardation was associated with nephron deficit, increased incidence of systemic hypertension and development of glomerulosclerosis in adulthood.[18–20] Above findings and those indicating impaired organogenesis, and reduced number of glomeruli are indicators of delelerious effect of CsA on developing kidney.

CONCLUSION

The developing kidney from foetuses of 12 dams on histological examination showed substantial decrease in their cortical width from 582.26±46.17 m to 530.71±31.69 m. The results clearly showed the deleterious effect of CsA on the developing kidney. Since the drug is very commonly used as an immunosuppressant in organ transplantation, therefore, further investigations are needed to confirm the findings in humans.

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