

Cyclosporin A Induced Tubulointerstitial Changes in Developing Kidney

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ABSTRACT

Purpose: To study tubulointerstitial changes in developing kidney by administering therapeutic doses of CsA to pregnant Albino mice throughout gestation.

Materials & methods: Twelve pregnant mice were divided into two groups, A and B, having six animals each. Cyclosporin (Sandimmun, Novartis, Switzerland) was freshly prepared in normal saline daily having a concentration of 1mg/ml 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 treated with the comparable volume of normal saline given subcutaneously for 18 days during gestation. The pregnant mice were sacrificed at the end of experimental period. The fetal kidneys were dissected, fixed in 10% formalin and the sections were stained with Hematoxylin and eosin (H & E) for general histological study, Periodic acid Schiff (PAS) collagen depositions and Masson's trichrome for demonstration of collagen depositions. These sections were evaluated for interstitial fibrosis and tubular atrophy using Calcineurin inhibitor toxicity (CNIT) Score.

Results: Our results clearly indicate that CsA is deleterious for developing kidney in the usual therapeutic doses. The work illustrated harmful effects of Cyclosporin leading to statistically significant histological changes including interstitial fibrosis, tubular atrophy suggestive of fetal nephrotoxicity.

Conclusion: The current study investigated the effects of CsA given to mice during gestation in therapeutic doses. The work clearly illustrated harmful effects of Cyclosporin leading to statistically significant histological changes including interstitial fibrosis, tubular atrophy suggestive of fetal nephrotoxicity and may eventually lead to renal failure. It might produce comparable effects in human conceptuses after intake of CsA by pregnant mothers.

Key words: Cyclosporin A, nephrotoxicity

INTRODUCTION

Cyclosporin (CsA) is one of the most potent immunosuppressants used for the management of multiple-organ transplantation. However, the clinical utility of CsA as an immunosuppressive agent has been significantly limited by the frequent occurrence of chronic CsA nephropathy, characterized by progressive renal insufficiency, arteriopathy, interstitial inflammation, and striped interstitial fibrosis^{1,2}. Two forms of CsA renal toxicity have been described: acute and chronic nephropathy. Acute nephrotoxicity, caused by renal afferent arteriolar vasoconstriction, is dose related³ and mainly occurs after prolonged treatment.⁴ Although these changes are reversible but their consequences remain a major barrier to long-term survival of the nephrotoxicity is characterized by reduced glomerular

filtration rate, pathological changes, such as proximal tubular swelling and necrosis, infiltration of macrophages and striped interstitial fibrosis; these changes are irreversible^{5,6}.

The mechanism of CsA-induced interstitial fibrosis, which can be patchy or striped^{7,8} has been attributed to an angiogenesis-dependent upregulation of molecules that promote scarring, such as TGF β ¹⁹ and osteopontin¹⁰. CsA may also impair the regenerative capacity of microvascular endothelial cells and induce apoptosis¹¹. Tubulointerstitial fibrosis is a common feature of progressive renal injury in almost all forms of renal diseases¹². It has been shown that tubulointerstitial injury is a more consistent predictor of functional impairment than glomerular damage¹³. Chronic inflammation generally precedes the development of fibrosis and inflammatory cytokines are important mediators of fibrogenesis¹⁴. Tubulointerstitial fibrosis is characterized by the destruction of renal tubules and interstitial capillaries as well as by the accumulation of extracellular matrix proteins, such as collagen types I, III, IV, V, VII, fibronectin and laminin¹⁵. Interstitial fibroblasts, myofibroblasts, tubules and

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inflammatory cells have all been identified as sources of the increased matrix protein production in renal fibrosis¹⁶. The nephrotoxicity of CsA among adults is well documented, however its effects on developing kidney remain to be established, therefore, the aim of the current work was to study interstitial changes in developing kidney by administering CsA (50mg/kg sc) to pregnant Albino mice throughout gestation.

MATERIAL & METHODS

Sixteen mice (6-8week old) weighing 25-30gm were used; comprising twelve females and four males. They were kept under standard condition of temperature ($24\pm 1^{\circ}\text{C}$) and humidity ($55\pm 5\%$) 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 mating¹⁷. When pregnancy was confirmed by vaginal plug, twelve pregnant mice were divided into two groups, having six animals each. The experimental group B was subjected to single daily subcutaneous injections of 50 mg/kg CsA (Sandimmun, Novartis, Switzerland) prepared in normal saline, for 18 days. The control group A 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 fetuses 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 albuminized glass slide before staining with Haematoxylin and Eosin (H&E) for histology, Periodic acid Schiff (PAS) stain for the demonstration of basement membranes and Masson's trichrome for demonstration of collagen depositions. The sections were evaluated for tubular atrophy and interstitial fibrosis using Calcineurin inhibitor toxicity (CNIT) score¹⁸.

The changes in interstitium and tubular structure of the kidneys were determined using a graticule provided with an eyepiece. The stained sections were studied under light microscope (Leica DM 1000) using X 10, X 20 and X 40 objectives. The interstitial fibrosis was evaluated at the corticomedullary junction, using point counting method and scored semiquantitatively as previously described^{7,7} Score 0: 0-5 % affected area; score 1: 6-25% affected area; score 2: 26-50% affected area; and score 3: >50% affected area.

Statistical analyses: Mean \pm SD were given for normally distributed quantitative variables, frequencies and percentages were given for

qualitative variables. Independent sample t test was applied to group differences. A "p" value of <0.05 was considered statistically significant.

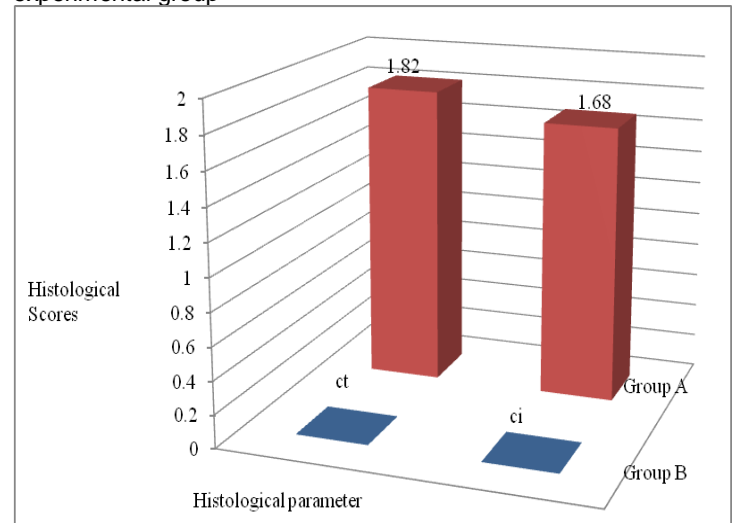
RESULTS

The interstitium in CsA treated group displayed striped fibrosis and tubular atrophy with preferential and early involvement of the medullary rays resulting in a band-like pattern. Striped fibrosis was identified as areas of interstitial fibrosis and tubular atrophy, alternating with preserved cortex localized mainly around the proximal tubules, extending from subcapsular cortex towards the medulla. In the treated group the interstitial fibrosis was observed at the corticomedullary junction as collagen deposition, tubular dilation and tubular atrophy (Fig.3).

Atrophic proximal tubules were disorganized, irregular in shape rested on thickened basement membrane and lacked brush borders (Fig.2). Dilated Proximal tubules were also observed with thickened basement membrane (Fig.2). The score for tubular atrophy was 1.82 ± 0.38 in the experimental group however the score was 0.00 ± 0.00 in control group respectively ($P>0.000$).

By Masson trichrome stain, an early development of fibrosis was demonstrated in the tubulointerstitium with in treated group (Fig 3) blue stain). Masson's trichrome staining in the treated group revealed atrophic tubules surrounded by a broad zone of matrix proteins most likely collagen fibers. (Fig.1). A significant increase in interstitial fibrosis score was also observed in the CsA group compared with the control group (1.8 ± 0.41 vs. 0.00 ± 0.00 , $P < 0.001$ Fig.1).

Fig.1: Comparison of histological scores in control and experimental group



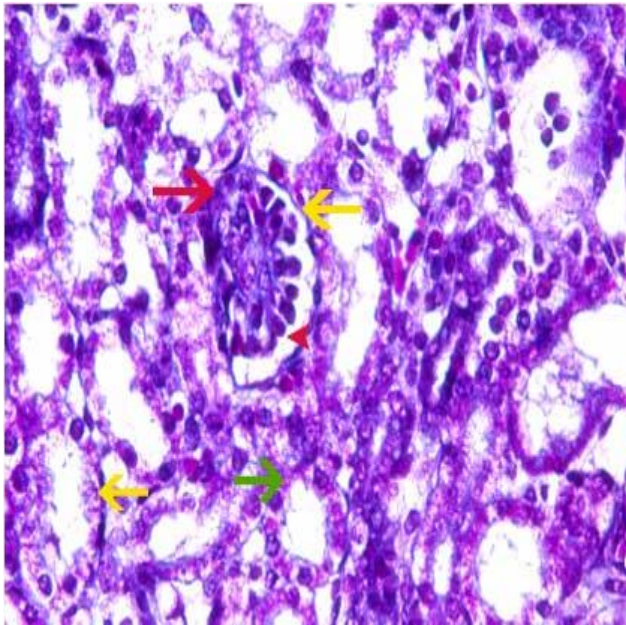


Fig. 1: Photomicrograph of renal cortex from control group A showing little amount of blue stained interstitium (yellow arrow) between different sections of renal tubules (green arrow). The adjacent interstitial spaces were visible and without inflammatory infiltrate. One renal corpuscle (red arrow) was looking normal with prominent urinary space (red arrow head) in the section. Masson's trichrome stain. X400.

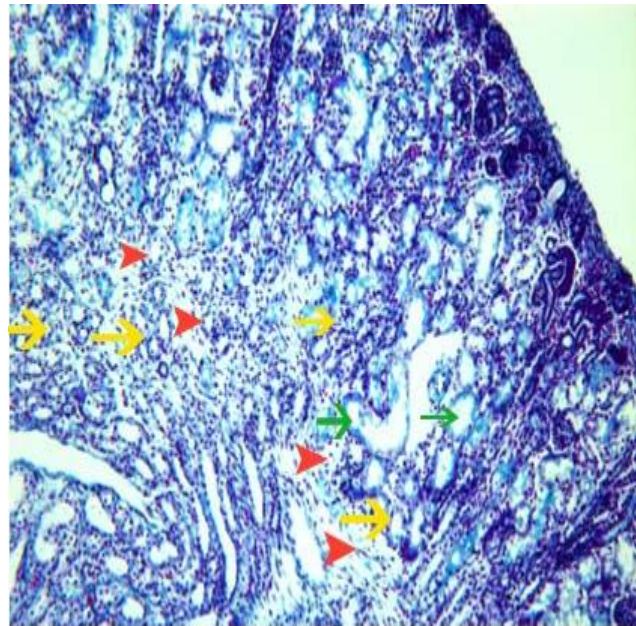


Fig.3. Photomicrograph of fetal kidney from group B showing multiple foci of atrophic tubules accompanied by patches of interstitial fibrosis in renal parenchyma. The atrophic tubules (yellow arrow) were seen surrounded by bluish green stained zones of fibrosis (red arrow head). The dilated tubules (green arrow) were also visible. Masson's trichrome. X 200.

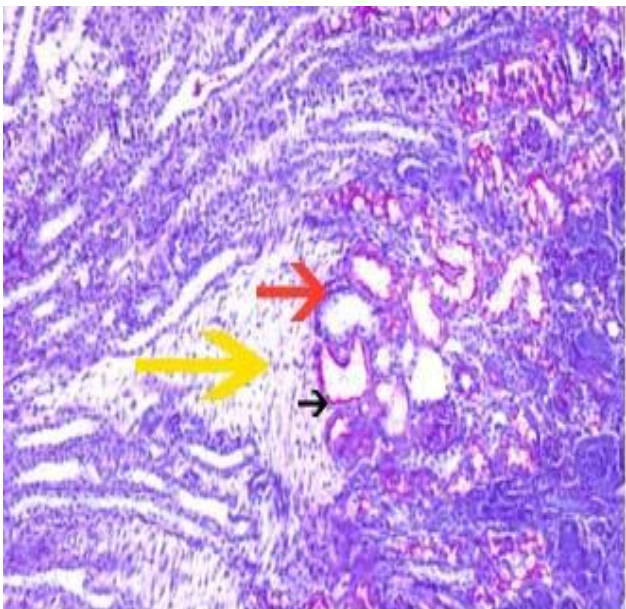


Fig.2. Photomicrograph of fetal kidney from experimental group B showing a large area of interstitial fibrosis (yellow arrow) at the corticomedullary junction. A large focus of atrophic tubules (red arrow) was visible; they were dilated and distorted with thickened basement membrane (black arrow). PAS stain. X100.

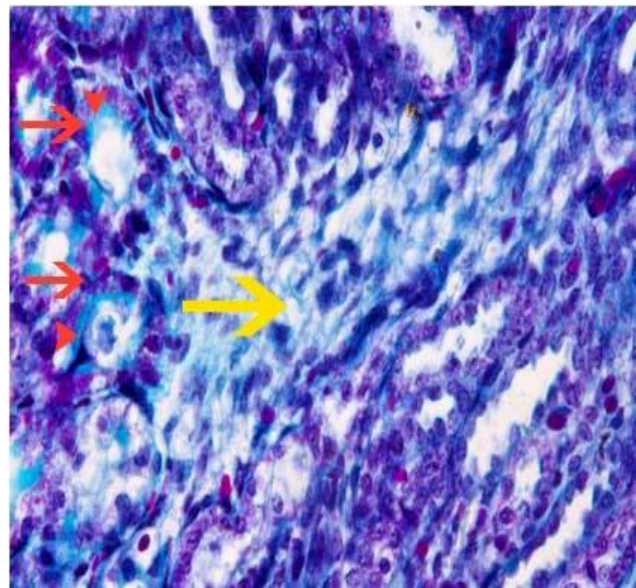


Fig.4. Photomicrograph of fetal kidney from CsA treated group B showing light green stained areas of fibrosis (yellow arrow) with moderate interstitial thickening between atrophic collecting tubules (red arrow). The areas of interstitial fibrosis appeared solid and deeply stained than normal interstitial tissues. The atrophic tubules were also showing bluish green stained areas of fibrosis (red arrow head). Masson's trichrome stain. X 400.

DISCUSSION

The introduction of Cyclosporin A (CsA) into clinical transplantation two decades ago as an immunosuppressive agent to prevent allograft rejection lead to a significant improvement in both allograft half-life and patient survival. Its usage in higher doses is however restricted due to its nephrotoxicity producing a spectrum of clinical manifestations from precipitous decrease in glomerular filtration rate (GFR) to chronically progressive scarring¹⁹.

CsA induced nephrotoxicity in our experimental model displays similar structural changes as observed earlier in the experimental model showed using adult rat, where in CsA treated animals showed typical histopathological features of chronic CsA nephrotoxicity including striped interstitial fibrosis, and tubular atrophy.^{20,21} The present study stipulates to evaluate the injurious effects of CsA in developing kidney when given in therapeutic doses to the mice.

Our results in CsA treated animals also showed tubular atrophy and striped interstitial fibrosis; these were statistically significant changes and in accord with the earlier work^{22,23}. The quantitative scoring in our investigation showed that CsA administration was associated with marked interstitial fibrosis when given in therapeutic doses to the mice.

Tubulointerstitial fibrosis is an important factor in the pathogenesis of chronic Cyclosporin nephropathy and considered as crucial determinants in progressive renal injury²⁴. The severity of tubulointerstitial fibrosis has long been considered as a crucial determinant of progressive renal injury²⁵. The previous studies demonstrate that its development is related to both reversible alterations and irreversible damage to all compartments of the kidneys, including glomeruli, arterioles, and tubulointerstitium and have shown that decrease in glomerular filtration rate is correlated with tubulointerstitial injury²⁶. Myers et al. were the first to demonstrate that Cyclosporin not only induces reversible alterations in renal vascular resistance, but is associated with irreversible damage of the renal architecture.²⁷ The interstitial fibrosis was patchy or "striped" in appearance; this was due to areas of interstitial fibrosis alternating with normal cortex; similar findings were reported earlier;²⁸ The tubules became even more distended, partly due to increasing tubulointerstitial fibrosis (Fig.3). An expansion of the cortical interstitium is highly correlated with tubular lesions, especially tubular atrophy. The widening of the interstitial space in chronic renal diseases is mainly due to increased extracellular matrix (ECM), and increased cellularity

(fibroblasts, macrophages, and lymphocytes) may also contribute to the tubulointerstitial fibrosis

There is an intimate relationship between interstitial fibrosis and tubular atrophy, both of which are closely correlated with the decline in renal function. The quantitative scoring in our investigations showed that CsA administration was associated with marked interstitial fibrosis (S=1.68) and tubular atrophy (S=1.82); both were recognized as an indicator of renal disease severity and its progression¹³.

REFERENCES

1. Rezzani R, Rodella FL, Tengattini S, Bonomini F, Pechanova O, Kojšová S et.al. Protective role of Polyphenols in Cyclosporin A-induced nephrotoxicity during rat pregnancy. *Journal of Histochemistry and Cytochemistry*.2006; 54(8):923-932.
2. Nishi S, Imai N, Alchi B et.al. The morphological compensatory change of peritubular capillary network in chronic allograft rejection. *Clin Transplant*. 2005; 19(14): 7-11.
3. English J, Evan A, Houghton DC, Bennett WM. Cyclosporin induced acute renal dysfunction in the rat. *Transplantation*. 1987; 44: 135-141.
4. Kahan BD. Cyclosporin. *N Engl J Med*. 1989; 321: 1725-1738.
5. Mayer RD, Berman S, Cockett AT, Maines MD. Differential effects of cyclosporin on hepatic and renal heme, cytochrome P-450 and drug metabolism. Possible role in nephrotoxicity of the drug. *Biochem Pharmacol*. 1989; 38: 1001-1007.
6. Nast CC, Adler SG, Artishevsky A, Kresser CT, Ahmad K, Anderson PS. Cyclosporin induces elevated procollagen alpha 1(1) mRNA levels in the rat renal cortex. *Kidney Int*. 1991; 39: 631-638.
7. Verpooten GA, Wybo I, Pattyn VM, Hendrix PG, Giuliano RA, Nouwen EJ et al. Cyclosporine nephrotoxicity: comparative cytochemical study of rat kidney and human allograft biopsies. *Clin Nephrol*. 1986; 25: 18-22.
8. Rezzani R, Rodella L, Corsetti G, Bianchi R Does methylene blue protect the kidney tissues from damage induced by cyclosporine A treatment? *Nephrol*. 2001; 89: 329-336.
9. Ling H, Li X, JHA S, et.al. Therapeutic role of TGF- β neutralizing antibody in mouse Cyclosporin A nephropathy: Morphologic improvement associated with functional preservation.2003; 14: 377-388.
10. Li C, Yang CW, Kim WY et al. Reversibility of chronic Cyclosporin nephropathy in rats after withdrawal of Cyclosporin. *Am J Physiol*. 2003; 284(2): 389-398.
11. Ahmad SS, Sitrobel HW, Napoli KI, Grevel J. Adrenochrome reaction implicates oxygen radicals in metabolism of Cyclosporin A and FK 506 in rat and human liver microsomes. *J Pharmacol Exp Ther*. 1993; 265: 1047-1054.
12. Cameron JS. Tubular and interstitial factors in the progression of glomerulonephritis. *Pediatr Nephrol* 1992 6: 292-303.

13. Risdon RA, Sloper JC, de Vardener HE. Relationship between renal function and histological changes found in renal biopsy specimens from patient with persistent glomerulonephritis. *Lancet* 1968; 2: 363-366.
14. Strutz F. Novel aspects of renal fibrogenesis. *Nephrol Dial Transplant* 1995; 10: 1526-1532.
15. Eddy AA. Molecular insights into renal interstitial fibrosis. *J Am Soc Nephrol*.1996 7: 2495-2508.
16. Alper CE, Hudkins kL,FloegeJ,Johnson RJ. Human renal cortical interstitial cells with some features of smooth muscle cells participate in tubulointerstitial and crescentic glomerular injury.*J Am Soc Nephrol* 1994 5: 201-209.
17. Venkataraman R, Koneru B, Wang CCP, Burckart GJ, Starzl TE. Cyclosporin and its metabolites in mother and baby. *Transplantation* 1988; 46:468-469.
18. Kambham N, Nagarajan S, Shah S, Li L, Salvatierra O, Sarwal MM. A novel semiquantitative clinically correlated Calcineurin inhibitor toxicity score for renal allograft biopsies. *Clin J Am Soc Nephrol*. 2007; 2: 135-142.
19. Myers BD, Sibley R, Newton L et.al. The long term course of cyclosporin associated chronic nephropathy. *Kidney Int*. 1988; 33: 590-600.
20. Buffoli B, Pechanva O, Kojsova S et.al. Proviron Prevents CsA-induced nephrotoxicity by reducing reactive oxygen Species, iNOS, and NF-kB expression.2005; 53:1459-1468.
21. Rezzani R, Rodella LF, Bianchi R. Early metabolic changes in peripheral blood cells of renal transplant recipients treated with cyclosporine A. *Int J Immunopharmacol*. 1999; 21:455-462.
22. Bianchi R. Induction of endothelin in rat kidney after cyclosporin A treatment. *Acta Histochem*. 2001; 103: 423-431.
23. Nath KA. Tubulointerstitial changes as a major determinant in the progression of renal damage. *Am J Kidney Dis* 1992;20: 1-17.
24. Naesens M, Kuypers DRJ, Minnie S. Calcineurin Inhibitor Nephrotoxicity.*Clin J Am Soc Nephrol* 4: 481-508, 2009.
25. Myers BD, Ross J, Newton L, Luetscher J, Perlroth M. Cyclosporin-associated chronic nephropathy. *NEJM*. 1984; 311: 699-705.
26. Liptak P, Ivanyi B. Primer: histopathology of calcineurin-inhibitor toxicity in renal allografts. 2006; 2: 398-404.
27. Nankivell BJ, Borrows RJ, Fung CL, O'Connell PJ, Allen R, Chapman JR. The natural history of chronic allograft nephropathy. *NEJM*. 2003; 349: 2326-2333.