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EFFECT OF CYCLOSPORINE WITHDRAWAL AFTER SHORT- AND LONG-TERM CYCLOSPORINE A EXPOSURE ON THE KIDNEY OF MALE ALBINO RATS

By

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INTRODUCTION

The cyclic fungal peptide, Cyclosporine A (CsA) is one of the most potent immunosuppressants used for the management of multiple-organ transplantation. CsA has improved early (1year) graft survival rates after kidney, heart, liver, pancreas and bone marrow transplantation and has contributed to ameliorate the treatment of autoimmune diseases refractory to conventional therapy **(Goral et al., 1997; Kagawa et al., 2003; Ziolkowski et al., 2003; Hesselink et al., 2004)** . The immunosuppressive effect of CsA is achieved through its impairment to interleukin-2 (IL-2) production resulting in inhibition of calcineurin that is involved in activation of T lymphocytes. Consequently, calcineurin inhibition blocks immune cell mediated reaction against the transplanted tissue **(Faulds et al.,1993)**. However, the introduction of this drug did not translate into improved long-term graft survival. There is considerable concern that one reason for this is that on the long-term, CsA nephrotoxic effects counteract the protective role of the drug as immunosuppressant **(Cattaneo et al., 2004)**.

The frequent occurrence of chronic CsA nephropathy, which is characterized by progressive histopathological lesions and renal insufficiency, has been significantly limited the clinical utility of CsA as an immunosuppressive agent **(Li et al., 2003; Nielsen et al., 2003; Cattaneo et al., 2004)**. However, **Bruke et al. (1994)** claimed that the majority of renal-transplant recipients tolerated long-term CsA therapy without evidence of progressive toxic nephropathy and the graft failure was most often due to rejection. On the other hand, **Bennett et al. (1996)** observed CsA-induced nephropathy in recipients of non renal allograft and after treatment of patients suffering from autoimmune diseases such as uveitis, diabetes mellitus type I, psoriasis, and rheumatoid arthritis where the cases were free from damaging effects on the kidney due to allograft rejection. The exact mechanism underlying chronic CsA nephropathy is poorly understood. However, using a well established animal model, a complex network including the rennin-angiotensin system **(Yang et al., 2001)**, nitric oxide **(Shihab et al., 2003)**, transforming growth factor-β1 **(Benigni et al., 1999)** and chemoattractant molecules **(Pichler et al., 1995)** has been shown to be involved.

Campistol and Sacks (2000) attributed the CsA-induced nephrotoxicity to the impairment of the calcineurin-activating IL-2, which could be associated with impairment of the transcription of many other genes encoding nitric oxide synthase, transforming growth factor-β, endothelin, and proteins implicated in cellular protection against apoptosis, triggering a sequence of undesirable events that would eventually lead to renal toxicity.

The effects of CsA dose reduction, or complete withdrawal of CsA, have been previously examined in clinical trials and experimental studies. However the results obtained are conflicting **(Kang et al., 2001; Weir et al., 2001).** Moreover, it is not clear in the clinical settings whether improved chronic allograft nephropathy is due to CsA dose reduction or elimination alone or the beneficial effects of other nonnephrotoxic immunosuppressive drugs used as a replacement therapy **(Weir et al., 2001)**.

The aim of this work is to study the possible histological changes and their reversibility in the kidney of adult male albino rats following short- and long term exposure to CsA using light and electron microscope so as to evaluate the efficacy of CsA withdrawal on the viability of the renal tissue.

MATERIALS AND METHODS

Forty Sprague-Dawley adult male albino rats, weighing 190 – 250 gm, were used in this study. The animals were kept in plastic cages in an air-conditioned animal house (temperature 22 ± 2°C) with optimal illumination cycle. The animals had free access to drinking water and a pellet diet and were divided into three groups as follows:

Group I (control group, N=20)**:** The animals were given a daily oral dose of 0.5 ml olive oil using a gastric tube. Then animals were killed at intervals of 3, 7, 9 and 13 weeks, 5 animals at a time.

Group II (CsA-exposed group; N=10): the animals of this group received a daily oral dose of CsA (15 mg/kg body weight) using a gastric tube. Neoral (oral formulation), which is a CsA for microemulsion manufactured by Novartis Pharmaceuticals (Basel- Switzerland) was dissolved in olive oil so as to give a final concentration of 3 mg/0.5ml. The animals were killed at intervals of 3 and 9 weeks, 5 animals at time.

Group III (CsA withdrawal group; N=10): the animals of this group received the same treatment as that of group II, then divided into two subgroups; group III-A (n=5) where the animals were exposed to the treatment for 3 weeks then killed after 4 weeks of drug withdrawal and group III-B (n=5) where the animals exposed to the treatment for 9 weeks then killed 4 weeks later.

All the animals were killed by over dose of ether and the kidneys were extracted.

Light microscopical study

A small piece from each kidney specimen was excised for ultrastructural study, and then the specimens were fixed in 10% formol saline and processed for paraffin blocks. Sections of 6μm in thickness were cut and stained with hematoxylin and eosin, Masson's trichrome **(Masson, 1924)** and periodic acid-Schiff **(PAS) (Bancroft and Gamble, 2002)** for histological and histochemical studies.

Electron microscopical study

The small pieces taken from the kidney specimens were immediately fixed in 4% gtuteraldehyde solution for 3 hours and then washed in phosphate buffer, post fixed in I% buffered osmium tetroxide for one hour, dehydrated and finally embedded in epoxy (Epon). Ultrathin sections 50-80 nm were contrasted with uranyl acetate **(Watson, 1958)** and lead citrate (**Reynolds, 1963)** and photographed with a Joel S_{100} electron microscope.

Histomorphometric quantification

Using the image-analyzer computer assisted by the software Leica Qwin 500 and its binary image with a standard measuring frame of 119616.7 μm^2, the following parameters were estimated:

1- The percentage of collagen fibers in the frame:

Area of collagen fibers/ total area of the field X 100.

2- The percentage of the glomerular damage :

Number of affected glomeruli/ total number of the glomeruli available in the field X 100

These data were measured, using a x 20 objective, in 10 fields of each specimen and the mean values were obtained. The results were subjected to statistical analysis and represented in histograms (figs. 1 ,2).

Statistical analysis

The Statistical Package for the Social Sciences (Student "t" test) was used in data analysis. Data were expressed as mean ± SE.

RESULTS

A-Statistical results of the histomorphometric study (Figs. 1,2)

The measurements of the mean percentage of collagen fibers as well as of the glomerular damage in long-term CsA exposed animals (9weeks) showed the highest values compared with other groups and such increase was statistically highly significant compared with those of the control group. Withdrawal of CsA after long-term exposure did not considerably reduce the values of these measurements, which showed a statistically highly significant increase, compared with the control group. Short-term CsA exposure showed also significant increase in the above-mentioned measurements, compared with those of the control. However, CsA withdrawal after short-term exposure markedly reduced the values of these measurements, which did not show significant changes compared with the control group.

B-Results of histological study

Group I (control group)

The light microscopical examination of the control kidney showed that it was formed of cortex and medulla. The cortex displayed numerous globular structures, the renal corpuscles, which were formed of dense rounded structures, the glomeruli. Each glomerulus was surrounded by a narrow Bowman's (urinary) space followed by a single layer of flat squamous cells, the Bowman's capsule lining the renal corpuscle. The glomerulus was formed of network of densely packed capillaries and numerous nuclei of capillary endothelium, mesangial cells and epithelial cells (podocytes). The cortex also showed the proximal convoluted tubules lined by cuboidal cells with granular acidophilic cytoplasm and apical brush border, the distal convoluted tubules lined by low cuboidal cells with rounded nuclei and the macula densa, which is a portion of distal convoluted tubules in contact with the renal corpuscle (fig.3). The renal medulla contained collecting tubules lined with simple cuboidal to low columnar cells as well as segments of the loop of Henle (fig.4).

The electron microscopical study of renal glomeruli demonstrated mesangial cells and discrete feet processes of the podocytes. The glomerular basement membrane showed its normal trilaminar appearance with uniform thickness (fig.5). The epithelial lining cells of proximal convoluted tubules demonstrated apical microvilli, a mid-positioned nucleus and abundant electron dense mitochondria. Some lysosomes and microvesicles as well as intact basal cell membrane could be seen (fig.6).

Group II (CsA-treated group)

*Three weeks (short-term) exposure:

Histological examination of the kidney specimens of the animals of this group showed changes in the renal corpuscles represented in proliferation of the parietal layer of the Bowman's capsule (proliferative glomerulonephritis) (Fig.7) and glomerular capillary congestion (Figs. 8,9) with hemorrhage in Bowman's space (Fig. 8). Bowman's space obliteration (Fig.9) or widening (Fig.10), as result of mesangial cell hyperplasia or shrunken glomeruli, respectively, was also observed. The renal tubules showed luminal cast formation (Fig.7) as well as vacuolization or complete degeneration of the tubular lining epithelial cells and their luminal exfoliation (Figs.7, 8). Tubular dilatation (Fig.10) and partial or complete loss of the apical brush border of some proximal convoluted tubules (Fig.11) were also detected . Massive interstitial cellular infiltration and vascular congestion (Fig.12) as well as thickening of the arterioles with vacuolization of its smooth muscle cells were demonstrated (Fig.13). Focal areas of increased interstitial fibrous tissue formation were detected (Fig.14).

Electron microscopical examination demonstrated irregular thickening of the glomerular basement membrane with loss of its trilaminar appearance (amorphous basement membrane) and occasional fusion of the foot processes of the podocytes (Fig.15). The proximal convoluted tubules either normal or their lining epithelial cells showed ultrastructural changes represented in destruction of the apical microvilli and dentation of the nuclear envelop. The cytoplasm showed mitochondria with unclear boundaries (amalgamated) and many lysosomes (Fig.16).

*Nine weeks (long-term) exposure

Light microscopical examination of the kidney specimens showed that many renal glomeruli were affected demonstrating partial or complete degeneration while others showed mesangial hyperplasia with narrowing of the capsular space. Proliferation of the parietal layer of the Bowman's capsule with increase in its thickness could be detected. Large number of renal tubules were affected; some were atrophic while others showed generalized vacuolization or complete degeneration of their lining epithelial cells (Fig.17). Many proximal convoluted tubules showed loss of their apical brush border (fig.18). Large areas, occupied by homogenous eosinophilic material with many dilated congested blood vessels and massive cellular infiltration separating the renal tubules apart, were revealed (Fig.19) in the deep cortex and in the medulla. There was marked thickening of the wall of the arterioles and vacuolization of its smooth muscle cells (Fig.20) as well as a diffuse and marked increase in interstitial fibrous tissue formation with

thickening of the tubular basement membrane, tubulo-interstitial fibrosis (Fig.21).

The electron microscopical examination demonstrated sites of markedly thickened amorphous glomerular basement membrane with loss of its trilaminar appearance and frequent fusion of the foot processes of the podocytes (Fig.22-a). Many proximal convoluted tubules were affected showing ultrastructural changes of their lining epithelial cells represented in deep invaginations of the nuclear envelop with its irregularity as well as chromatin margination (apoptosis). The cytoplasm showed loss of differentiation of the cytoplasmic organelles at the apical part (apical necrosis) and basal accumulation of electron translucent mitochondria with loss of cristae and unclear boundaries (degeneration) as well as destruction of the finger like processes of the apical microvilli. Focal destruction of the basement membrane with partially vacuolated myelin figures containing basement membrane materials could be seen (Fig.22-b).

Group III (CsA-withdrawal group)

Group III-A (CsA-withdrawal following short-term exposure)

Four weeks after withdrawal of CsA, the kidney specimens showed that most of glomeruli and renal tubules were normally appeared (Fig.23). The brush border of most of proximal convoluted tubules was restored (Fig.24). No mesangial hyperplasia was detected. Few congested blood vessels were encountered (Fig.23) with subsidence of the interstitial cellular infiltration.

The electron microscopical examination of the glomeruli and proximal convoluted tubules are similar to those of the control group.

Group III-B (CsA-withdrawal following long-term exposure)

After four weeks of CsA withdrawal, the kidney specimens showed some normal glomeruli as well as those with variable degrees of degeneration and thickened Bowman's capsule. Renal tubules lined with degenerated epithelial cells were frequently encountered. There was a persistence of interstitial vascular congestion (Fig.25) with subsidence of the cellular infiltration. Thickening of the tubular basement membrane as well as persistent increase in the interstitial fibrous tissue formation was demonstrated (Fig.26).

The ultrastructural changes of the glomeruli and the proximal tubules are similar to those of long term CsA-exposure group.

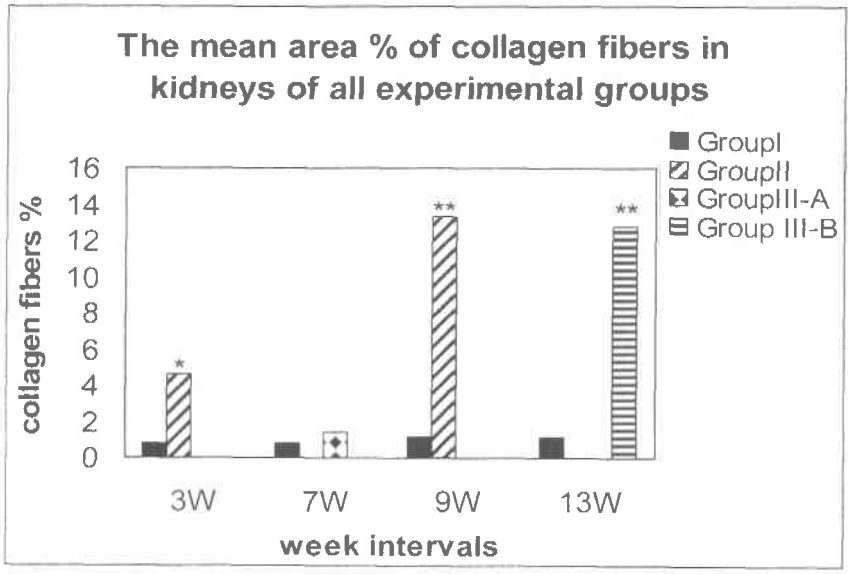


Fig. (1): A histogram showing the mean area percentage of the collagen fibers in the kidneys of all experimental groups.
*: significant with respect to the control group (P< 0.05)
**: highly significant with respect to the control group (P< 0.01)

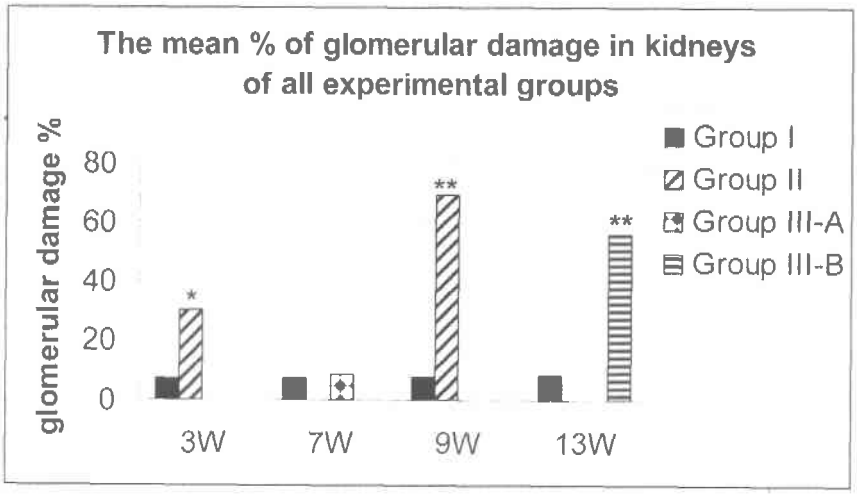
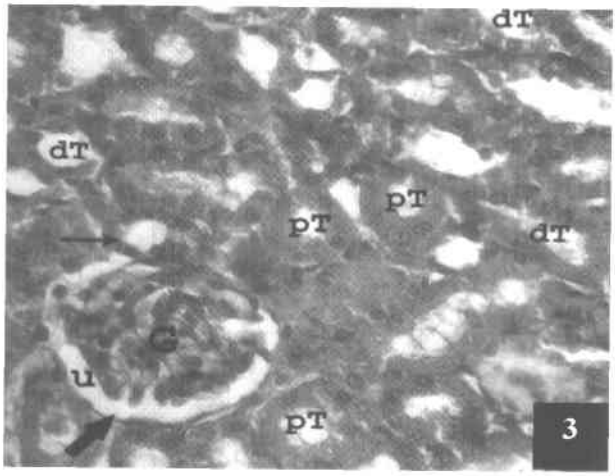
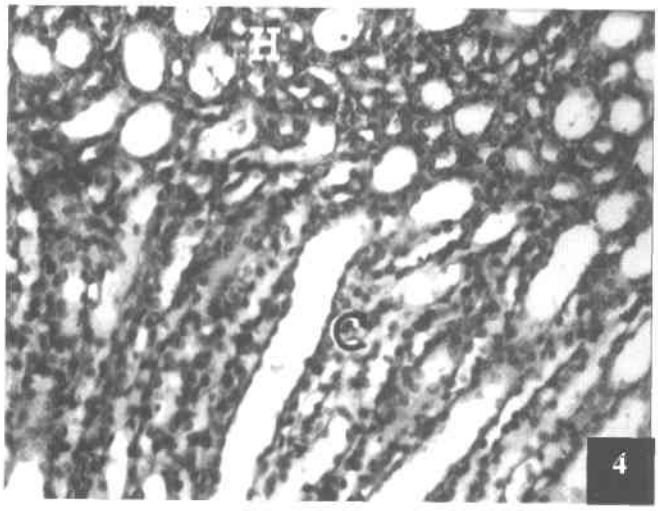


Fig. (2): A histogram showing the mean percentage of the glomerular damage in the kidneys of all experimental groups.
*: significant with respect to the control group (P< 0.05)
**: highly significant with respect to the control group (P< 0.01)



Figs. (3): A photomicrograph of a cross section of a kidney specimen of the control group showing renal cortex demonstrating renal corpuscle formed of a glomerulus (G) surrounded by urinary space (U) and lined by flattened squamous cells (thick arrow). Note the macula densa (thin arrow), the proximal convoluted tubules (pT) and the distal convoluted tubule (dT) separated by delicate interstitium. (Hx. & E.; x400)



Figs. (4): A photomicrograph of a cross section of a kidney specimen of the control group showing renal medulla with collecting tubules (C) and segments of loops of Henle (H). (Hx. & E.; x300)

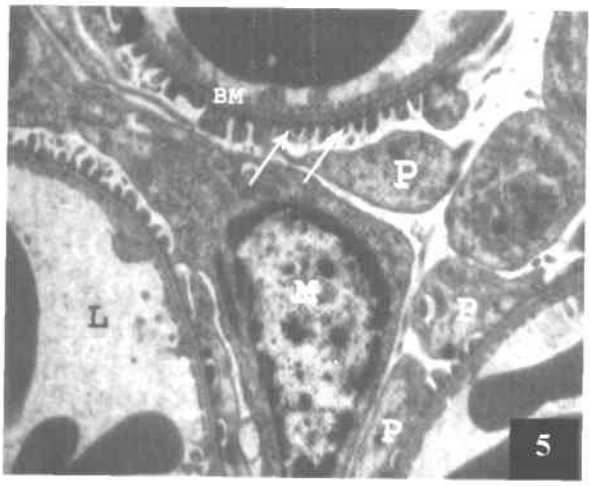


Fig. (5): An electron micrograph of a glomerulus of a kidney specimen of the control group showing mesangial cell (M) and discrete feet processes (long arrows) of the podocytes (P). The glomerular basement membrane (BM) shows its normal trilaminar appearance with uniform thickness. Note the lamina fenestrate (short arrows) lining the capillary (L). (x 4,600)

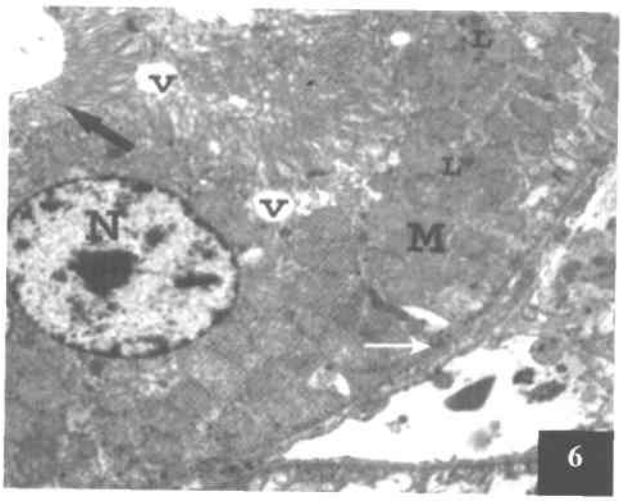


Fig. (6): An electron micrograph of part of proximal convoluted tubule of a kidney specimen of the control group showing apical microvilli (thick arrow), a mid-positioned nucleus (N), abundant electron dense mitochondria (M), some lysosomes (L) and microvesicles (v). Note the intact basal cell membrane (thin arrow). (x 3,600)

Plate I: Photomicrographs of cross sections of kidney specimens of group II, following 3 weeks of CsA exposure, showing:

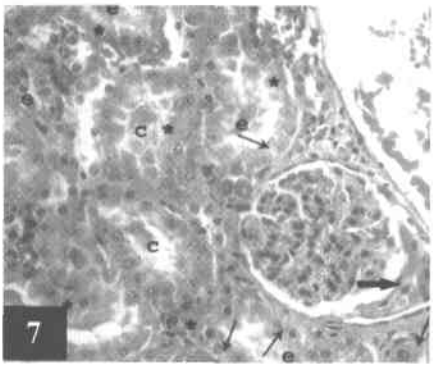


Fig. (7): Proliferation of the parietal layer of the Bowman's capsule (thick arrow). The renal tubules demonstrate cast formation (c) as well as vacuolization (thin arrows) and complete degeneration (*) of the lining epithelial cells with their luminal exfoliation (e). (Hx. & E.; x400)

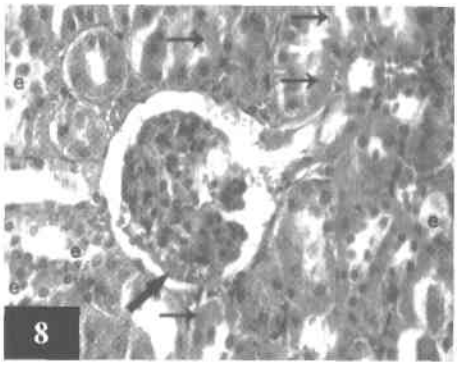


Fig. (8): Glomerular capillary congestion with hemorrhage in the Bowman's space (thick arrow). Degeneration of the lining epithelial cells (thin arrows) of the renal tubules and their exfoliation (e) in the tubular lumen can be seen. (Hx. & E.; x400)

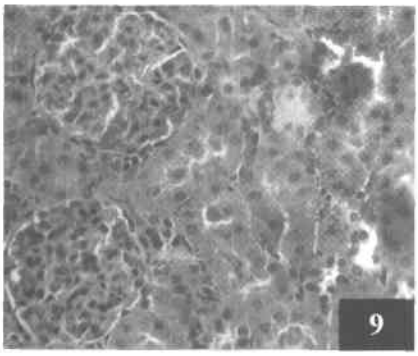


Fig. (9): Glomerular capillary congestion and mesangial cell hyperplasia with obliteration of the Bowman's space. (Hx & E.; x400)

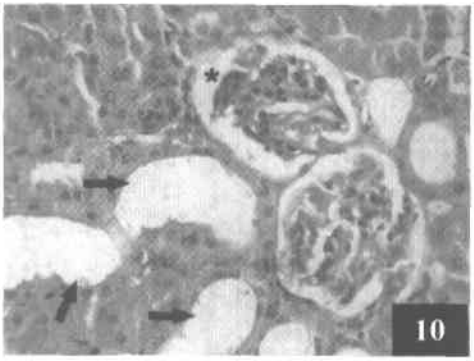


Fig. (10): Shrunken glomeruli with widening of Bowman's space (*). Note the tubular dilatation (arrows). (Hx. & E.; x400)

Plate II: Photomicrographs of cross sections of kidney specimens of group II, following 3 weeks of CsA exposure, showing:

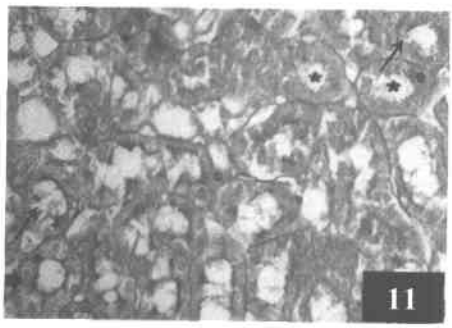


Fig. (11): Partial (long arrows) or complete loss (*) of the apical brush border of some of the proximal convoluted tubules. Note the tubule with intact brush border (short arrow).(PAS; x 400)

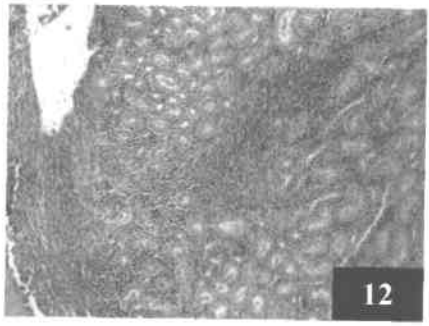


Fig. (12): Massive interstitial cellular infiltration and vascular congestion. (Hx. & E.; x 200)

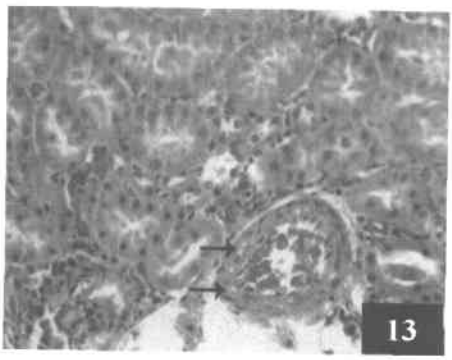


Fig. (13): A renal arteriole with thickening of its wall and vacuolization of its smooth muscle cells (arrows). (Hx. & E.; x 400)

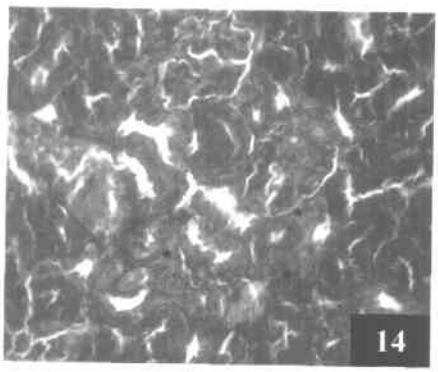


Fig.(14): Focal areas of increased interstitial fibrous tissue formation (*). (Masson's trichrome; x 300)

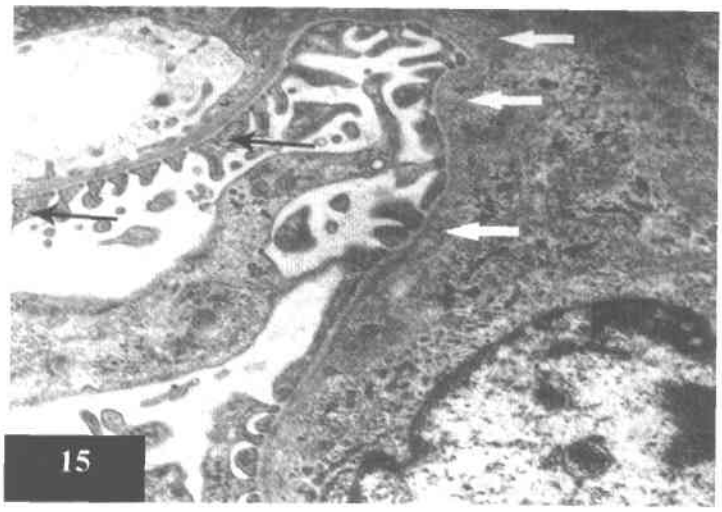


Fig. (15): An electron micrograph of kidney specimen of group II, following 3 weeks of CsA exposure, showing part of a glomerulus demonstrating irregular thickening of the glomerular basement membrane (thich arrows) with loss of its trilaminar appearance and occasional fusion of the foot processes of the podocytes (thin arrows). (x 5,400)

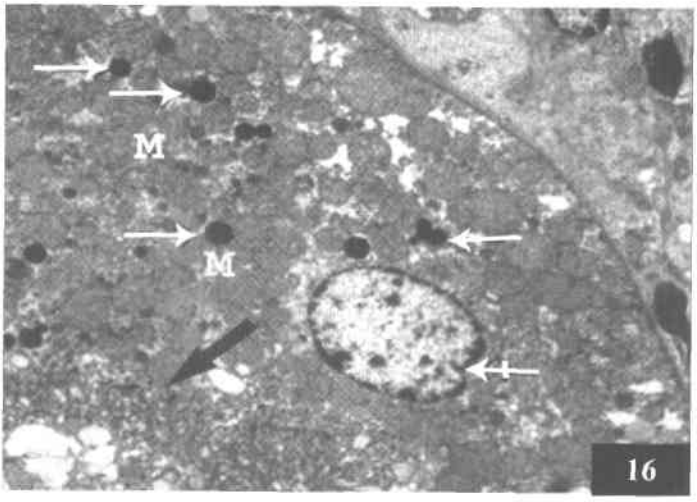


Fig. (16): An electron micrograph of kidney specimen of group II, following 3 weeks of CsA exposure, showing part of a proximal convoluted tubule demonstrating destruction of the apical microvilli (thick arrow), dentation of the nuclear envelop (crossed arrow). The cytoplasm shows mitochondria (M) with unclear boundaries (amalgamated) and many lysosomes (thin arrow). (x 2, 200)

Plate III: Photomicrographs of cross sections of kidney specimens of group II, following 9 weeks of CsA exposure, showing:

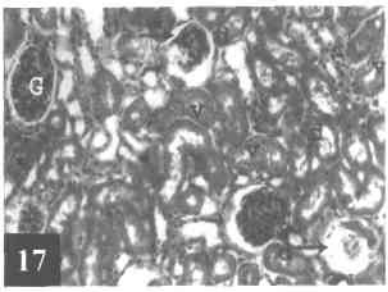


Fig. (17): Partially or completely degenerated glomeruli (long arrows) with thickening of the parietal layer of the Bowman's capsule (short arrows). A glomerulus (G) with mesangial hyperplasia as well as atrophic tubules (*) and those showing generalized vacuolization (v) or complete degeneration (d) of their lining epithelial cells can be seen. (Hx. & E.; x300)

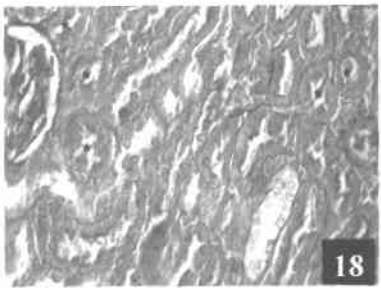


Fig. (18): Loss of the apical brush border of many proximal tubules (*) (PAS; x400)

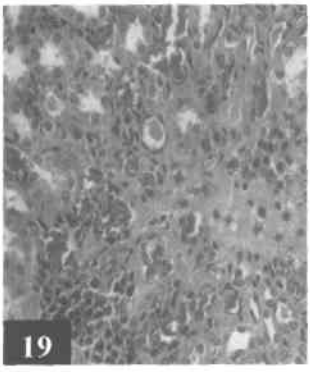


Fig.(19): Large area, occupied by homogenous eosinophilic material with many dilated congested blood vessels and massive cellular infiltration separating the renal tubules apart. (Hx. & E.; x400)

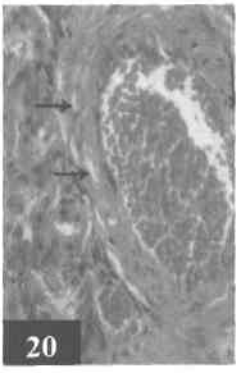


Fig. (20): Marked thickening of the wall of a renal arteriole with vacuolization of its smooth muscle cells (arrows). (Hx & E.; x400)

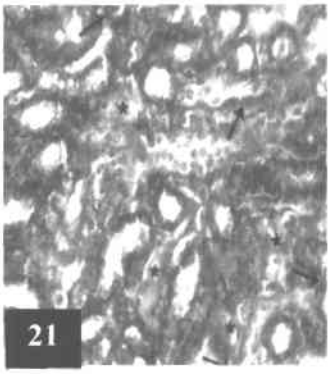
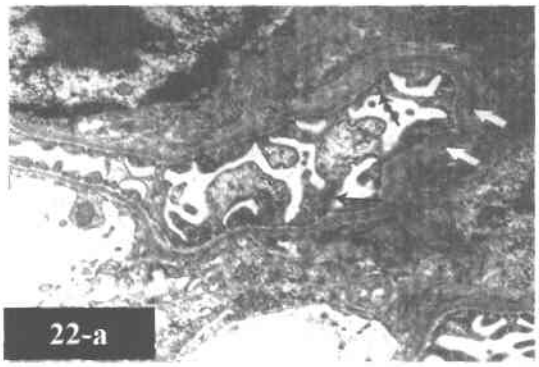
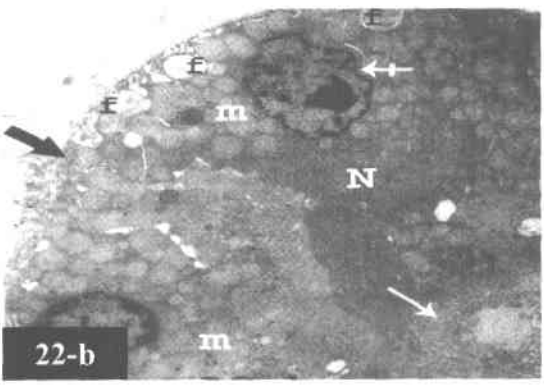


Fig. (21): Marked increase in interstitial fibrous tissue formation (*) with thickening of the tubular basement membrane (arrows). (Masson's trichrome; x 400)

Fig.(22-a,b): Electron micrographs of kidney specimens of group II, following 9 weeks of CsA exposure, showing:



(22-a): Part of a glomerulus demonstrating sites of markedly thickened glomerular basement membrane (thick arrows) with loss of its trilaminar appearance and frequent fusion of the foot processes of the podocytes (thin arrows). (x 5,400)



(22-b): Part of a proximal convoluted tubule demonstrating deep invaginations of the nuclear envelop (crossed arrow) with chromatin margination. The cellular cytoplasm shows basal accumulation of electron translucent mitochondria (m) with unclear cristae. Apical necrosis (N) with destruction of the finger like processes of the microvilli (thin arrow) can be also seen. Note the focal destruction of the basal cell membrane (thick arrow) with the partially vacuolated myelin figures containing basement membrane materials (F). (x 2,200)

tion of CsA. This implies that the histological changes in acute nephrotoxicity, as in case of **Prévot's** study, are quite different from those of chronic CsA nephrotoxicity.

In the present study, the increased interstitial fibrous tissue formation was detected in the kidney specimens of both short- and long-term CsA treated animals. It was focal in short-term exposure and diffused or segmental in long-term one with thickening of the tubular basement membrane forming tubulo-interstitial fibrosis. The diffuse tubulo-interstitial fibrosis was similarly reported by **Busauschina et al. (2004) and Fellstrom (2004)** after long term-CsA exposure (15 mg/ kg). On the other hand, **Satyanarayana and Chopra (2002)** exposing the animals to a short-term of CsA treatment (3weeks), demonstrated the same finding when they used a higher dose of CsA (20 mg/kg). This leads to the assumption that the diffused tubulo-interstitial fibrosis induced by CsA treatment is a dose and duration dependent. In contrary, **Disel et al. (2004)** did not record any increase in the interstitial fibrous tissue formation in rat kidneys after long-term CsA treatment of a dose of 15mg /kg, in spite of the marked changes in the renal tubules and interstitium. However, there is no apparent explanation for this contradiction.

The data obtained from the histological and histomorphometric study of the current work pointed to the crucial role of the duration of CsA exposure in determination the degree and severity of the renal tissue damage. In agreement, **Falkenhain et al. (1996) Hansen et al. (1998) and Jankauskiene et al. (2001)** revealed that with increasing time of exposure to CsA, even to low doses, there was a progressive increase in renal arteriolopathy as well as glomerular and tubular damage. The authors concluded that the renal damage increased in relation to time of CsA exposure and to the total dose of CsA that the patients received. Therefore, **Jankauskiene et al. (2001)** emphasized the importance of discontinuity of CsA treatment in case of development of nephrotoxicity rather than reduction of the CsA dosage.

The present work revealed marked affection of the proximal convoluted tubules, in CsA treated animals, represented in degeneration of their lining epithelial cells that showed apical necrosis, loss of their brush border, degeneration of the mitochondria, abundant lysosomes and focal destruction of their basement membrane. Comparable findings were presented by **Stacchiotti et al. (2002)** who added that these changes occurred even if CsA administrated at therapeutic dose. Furthermore, different studies reported that proximal tubules are the target of CsA **(Van de Water et al., 1994)** and the site of production of apoptotic genes **(Shihab et al., 1999). Stacchiotti et al. (2002)** assumed that the apoptotic or necrotic nuclei and detachment of brush border of the proximal tubules indicated a functional impairment of the urinary reabsorption. Such assumption is supported by

Heering et al. (1993) who revealed that the decrease in glomerular filtration rate and renal perfusion during chronic treatment with CsA was accompanied by a reduced proximal tubular capacity and concluded that functional parameters of the proximal tubular system can be used as indicators of CsA induced nephrotoxicity.

However, the reflection of renal structural changes on impairment of renal function is a point of controversy. **Satyanarayana and Chopra (2002) and Shi et al. (2004)** working on experimental animals demonstrated that CsA induced both histological changes as well as functional alterations of rat kidney. On the other hand, **Falkenhain et al. (1996)** concluded that there was no significant relationships between severity of renal pathological changes and renal functional parameters. The authors attributed that to the compensatory hyperperfusion of remaining healthy glomeruli preventing the pathological lesion from being reflected on kidney function until tissue damage become extensive. The view of **Falkenhain et al. (1996)** could explain the presence of chronic pathologic alterations in renal biopsies from CsA-treated patients who showed normal renal functions all the times of the biopsies **(Bennett et al., 1996)**. **Seron and Moreso (2004)** concluded that histological study of renal biopsy might have an important role in defining the type and degree of nephrotoxicity that could develop in patients receiving long-term CsA therapy as kidney function parameters did not precisely reflect the progression of chronic nephropathy.

The exact mechanism underlying chronic CsA nephrotoxicity is poorly understood. The osteopontin (OPN) expression, and the TGF-beta1, located in the interstitial macrophages, are chemoattractant phosphoproteins involved in inflammatory cell infiltration **(Pichler et al., 1995)**. They have been proposed to be important players in the development of tubulointerstitial injury, which follows the interstitial inflammatory events in chronic CsA nephropathy **(Kelly et al., 2002; Li et al., 2003; Li et al., 2004)**. Moreover, **Yang et al. (2001)** revealed that angiotensin II that is a potent growth factor inducing fibroblasts activation and tissue scaring, could have an important role in CsA-induced chronic nephrotoxicity where its chronic infusion in rat induced tubulointerstitial injury similar to that following chronic CsA administration. In the present work, the hyperplasia of mesangial cells, which are an extension to the juxta glomerular cells concerned with *rennin-angiotensin* system, and the massive renal interstitial cellular infiltration followed by diffused tubulointerstitial fibrosis in longer duration of CsA exposure suggest the interaction of all of the above-mentioned mechanisms in developing renal damage. Such suggestion is in a full agreement with the conclusion reached by **Vitko and Viklicky (2004)** and **Lee et al. (2004)** who demonstrated that CsA enhanced the activity of many cytokines including angiotensin II, TGF- beta1, chemoattractant osteopontin and vascular endothelial growth factor expressions together with down-regulation of nitric oxide synthesis. There is also growing evidence

suggesting the participation of free radicals in CsA nephrotoxicity **(Anjaneyulu et al., 2003; Parra et al., 2003; Disel et al., 2004)**. However, the prophylactic effect of the antioxidants needs further studies.

The effect of CsA dose reduction or its complete withdrawal, have been previously examined in clinical trials and experimental studies **(Kang et al., 2001; Weir et al., 2001; Yang et al., 2002; Li et al.,2004)**. However, the efficacy of CsA withdrawal on the progression of chronic CsA-induced fibrosis remains controversial. The study of **Franceschini et al. (1998)** demonstrated failure of CsA withdrawal, for 8 weeks, in reversing tubulointerstitial fibrosis induced by chronic CsA administration. However, **Yang et al. (2002)** found that 5 weeks of CsA withdrawal was effective in reversing the progression of tubulointerstitial fibrosis after short-term (4 weeks) administration of a relatively low dose of CsA (7.5 mg/Kg/day). These findings raise the possibility that the effectiveness of CsA withdrawal on the reversibility of the induced nephrotoxicity is dependent on CsA dosage and the duration of its exposure. Comparable findings can be deduced from the present work, which revealed reversibility of the renal structural changes, only, after short-term of CsA treatment. In agreement, many studies demonstrated that reversibility of the structural and functional changes of chronic CsA induced nephrotoxicity achieved when CsA withdrawal occurred early after diagnosis of nephropathy **(Li et al., 2003; Oberbauer et al., 2003; Stallone et al., 2003)** while prolonged administration of CsA could lead to chronic form of renal damage that potentially progress irreversibly to end-stage renal disease **(Busauschina et al., 2004; Cattaneo et al., 2004)**. Consequently, **Oberbauer et al. (2003)** and **Stallone et al. (2003)** recommended early CsA withdrawal as a safe option for recovery of the renal histological changes.

In conclusion, CsA administration results in histological changes in the rat kidney and the severity of these changes depends on the duration of CsA exposure. CsA withdrawal following short-term administration results in reversibility of the renal tissue alterations while withdrawal after prolonged exposure achieves minimal improvement with persistence of most of the pathological changes. Regular renal biopsies from patients receiving long-term CsA therapy are recommended for early diagnosis of nephrotoxicity, which then needs early drug withdrawal for better chance of complete recovery.

SUMMARY

Forty adult male albino rats were used in this study. They were divided into three groups; group I (control group) received daily oral dose of olive oil (0.5ml) and killed at intervals of 3,7,9 and 13 weeks, group II treated daily by an oral dose of cyclosporine A (CsA; 15mg/kg), then, killed at intervals of 3 and 9 weeks and group III-A and B where the animals re-

ceived the same treatment of group II for 3and 9 weeks, respectively, then were killed 4 weeks after drug withdrawal. The kidney were extracted from the killed animals and were processed for both light and electron microscopical studies.

The histomorphometric study revealed that the mean percentage of collagen fibers as well as of the glomerular damage in the renal tissues showed increases in their measurements in CsA exposed animals with their highest values in long-term CsA exposed ones, which were highly significant compared with the control and CsA withdrawal after long-term exposure did not considerably reduce the values of these measurements. On the other hand, drug withdrawal after short-term exposure did not showed significant changes in the values of the above-mentioned measurements compared with the control.

The light microscopical study showed that treatment with CsA resulted in histological alterations of the renal tissues, where the severity of which was duration dependent, and represented in mesangial hyperplasia, glomerular degeneration, thickening of the parietal layer of Bowman's capsule and tubular epithelial cell degeneration with tubular atrophy. The interstitial congestion, cellular infiltration, and fibrous tissue formation as well as thickening of the wall of the arterioles were also encountered. The electron microscopical study of the glomeruli revealed fusion of the foot processes of the podocytes and sites of thickening of the glomerular basement membrane with loss of its trilaminar appearance while the proximal tubules demonstrated sites of destruction of the basement membrane, irregular nuclear envelop, basal accumulation and degeneration of the mitochondria and apical cell necrosis with destruction of the apical microvilli. CsA withdrawal after short-term exposure resulted in histological recovery of renal tissue apart from sites of interstitial congestion while its withdrawal after long-term exposure led to minimal improvement with persistence of most of the histological changes.

In conclusion, CsA administration results in histological changes in the rat kidney and the severity of these changes depends on the duration of CsA exposure. CsA withdrawal following short-term administration results in reversibility of the renal tissue alterations while withdrawal after prolonged exposure leads to persistence of the pathological changes. Regular biopsies from chronically CsA-treated patients is recommended for better prognosis of cases developing CsA-nephrotoxicity .

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الملخص العربى

تأثير توقف التعرض للسيكلوسبورن (أ) بعد استخدامه لمدى قصير ومدى طويل على كلية ذكور الفئران البيضاء

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استخدم في هذا البحث أربعون فأر ذكر بالغ. وقد تم تقسيم الحيوانات الى ثلاثة مجموعات؛ المجموعة الأولى (المجموعة الضابطة) وقد تناولت يوميا عن طريق الفم جرعة من زيت الزيتون (٠,٥ ميلي) وقد تم قتل الحيوانات على فترات بعد٣ و٧ و٩ و١٣ اسبوع والمجموعة الثانية وقد عولجت الحيوانات فيها يوميا بجرعة من السيكلوسبورن (١٥ مجم / كجم من وزن الجسم) أُعطيت عن طريق الفم ثم تم قتلهم على فترات بعد٣ و٩ أسابيع والمجموعة الثالثة أ و ب حيث تناول فيها الحيوانات نفس علاج المجموعة الثانية لمدة ثلاثة وتسع أسابيع، على التوالي، ثم تم قتلهم بعد أربع أسابيع من توقف إعطاء الدواء. وقد تم استئصال الكلى وتحضيرها للدراسة بالميكرسكوب الضوئي والاكتروني.

وقد كشفت الدراسة الهيستومورفومترية ان متوسط نسبة ألياف الكولاجين و كذلك متوسط نسبة اصابة الكبيبات الكلوية قد أظهرت زيادة في قياساتها في الحيوانات المعرضة للسيكلوسبورن مع أعلى قيم لها ذي دلالة إحصائية عالية في الفئران التي تعرضت للسيكلوسبورن لفترة طويلة وذلك بالمقارنة بالمجموعة الضابطة وأن توقف إعطاء الدواء بعد التعرض له لفترة طويلة لم يقلل القيم هذه القياسات الى حد بعيد بينما توقف إعطاء الدواء بعد التعرض له لفترة قصيرة لم يؤد الى وجود تغيرات ذي دلالة إحصائية في القياسات السابق ذكرها بالمقارنة بالمجموعة الضابطة.

وقد أوضحت الدراسة بالميكروسكوب الضوئي أن العلاج بالسيكلوسبورن نتج عنه تغيرات هيستولوجية للأنسجة الكلوية والتي اعتمدت في حدتها على فترة التعرض للدواء

وتمثلت في زيادة الخلايا البلعمية وانحلال للكبيبات الكلوية وزيادة في سمك الطبقة الخارجية لكبسولة بومان وانحلال الخلايا المبطنة لجدار الأنبيبات الكلوية مع ضمور هذه الأنبيبات. وقد لوحظ أيضا وجود احتقان وارتشاح خلوي بين الأنسجة الكلوية مع تكون أنسجة ليفية وكذلك زيادة في سمك جدار الشريّينات. وقد أظهرت الدراسة الالكترومیكروسكوبية للكبيبات الكلوية التحام الزوائد القدمية للخلايا ذات الأقدام ومواضع تثخن في الغشاء القاعدي للكبيبات الكلوية مع فقدان المظهر ثلاثي الصفيحات لها، بينما أظهرت الأنبيبات الملتفة القريبة مواضع هدم للغشاء القاعدي للخلايا المبطنة لها وعدم انتظام الغلاف النووى وتجمع قاعدي وانحلال للمبتوكوندريا وتكرز قمي للخلايا مع تهتك الخميلات السطحية. وقد نتج عن توقف التعرض للسيكلوسبورن بعد استخدامه لفترة قصيرة إلى الشفاء الهيستولوجي للأنسجة الكلوية فيما عدا مواضع للاحتقان بين الأنسجة بينما توقف التعرض للدواء بعد استخدامه لفترة طويلة أدى إلي تحسن قليل مع استمرار معظم التغيرات الهيستولوجية.

ونستخلص من هذه الدراسة أن التعرض لمادة السيكلوسبورن يؤدي إلى تغيرات هيستولوجية للأنسجة الكلوية للفأر وأن خطورة هذه التغيرات تعتمد على طول فترة التعرض للدواء. وأن توقف استخدام السيكلوسبورن بعد التعرض له لأمد قصير يؤدي الى انعكاسية هذه التغيرات بينما توقف استخدام الدواء بعد طول مدة التعرض له يؤدي الى استمرار التغيرات الباثولوجية. وينصح بأخذ عينات بطريقة منتظمة للدراسة الهيستولوجية من كلية المرضى التي تتعرض لعلاج طويل الأمد بالسيكلوسبورن حتى يكون هناك تكهن أفضل لحالات التسمم كلوي الناتجة عن هذا الدواء.