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THE EFFECT OF CYCLOSPORIN-A ON THE HISTOLOGICAL STRUCTURE OF THE RAT KIDNEY

By

Mostafa M. El-Naggar and Ahmed A. Elayat

Department of Anatomy, Faculty of Medicine and Allied Sciences, King Abdulaziz University, Jeddah, Kindom of Saudi Arabia

ABSTRACT

Cyclosporin A (CsA) has proved its efficiency as a very potent immunosuppressive agent which have made it the drug of choice in many transplantation procedures. Following its clinical and experimental use, nephrotoxicity was reported as a major complication. Despite the proven effect of CsA on the function of the kidney, the morphological changes of nephrotoxicity that have been reported are conflicting and largely nonspecific. The present study was, therefore, undertaken to examine the structural changes of the kidney in CsA-treated rats.

Two groups of rats were injected subcutaneouly with CsA at doses of 10 and 50 mg/kg/day, respectively. A third group was injected daily with a similar volume of the vehicle with saline alone, and used as a control. Kidneys were processed for paraffin sections 1, 2 and 3 weeks after starting the injection and slides were stained with Hx. & E., PAS and Van Giesson. Histological changes were seen in scattered proximal convoluted tubules (PCT), especially those mostly located at the juxtamedullary cortex. Their cells appeared slightly swollen and the cytoplasm showed vacuolar spaces, 1 week after injection with CsA 10 mg/kg/day. Vacuolation of cells was seen in more tubules 2 and 3 weeks after injection of CsA 10 mg/kg/day, with occasional areas of increased cellularity, mostly lymphocytes and macrophages. Flattening of cells in some of the PCTs with widening of their lumen could be seen 3 weeks after CsA. Similar changes were seen in the kidneys of the rats which were treated with CsA 50 mg/kg/day, the changes, however, apparently affected a larger area and were prominent 3 weeks after CsA, where the tubules showed large vacuolar spaces and dark pyknotic nuclei.

Renal glomeruli of the CsA-treated rats did not show any apparent histological changes. Morphometric analysis showed a statistically significant decrease in the mean diameter of the glomeruli 1 week after treatment with 10 mg CsA ($P < 0.001$), followed by slight increase in their mean diameter at 2 and 3 weeks post-treatment. Their diameters were still, however, significantly lower compared to that of the control rats. Similar changes were noticed in the diameters of the glomeruli of the rats treated with 50 mg CsA, however, they returned nearly to their normal size

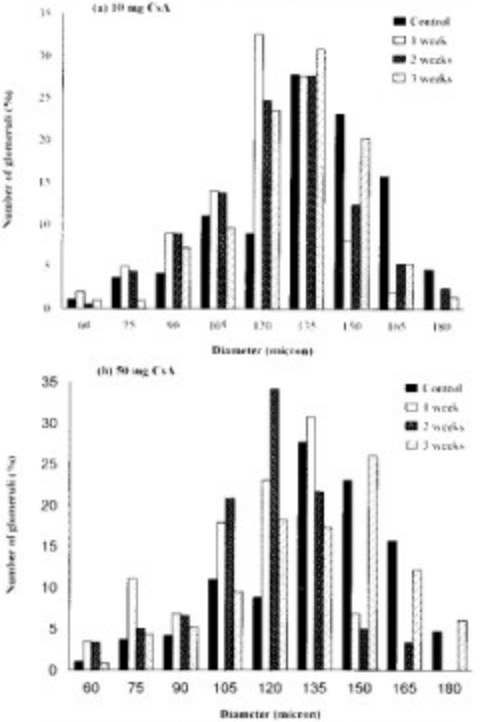

Fig. 8: Frequency distribution of the profile diameters of glomeruli of kidneys of:
a) rats injected with CsA 10 mg/kg/day, for 1, 2 and 3 weeks.
b) rats injected with CsA 50 mg/kg/day, for 1, 2 and 3 weeks.

serum creatinine concentration, which may be corrected by withdrawal of the drug or diminution of its dose *(Powles et al., 1980; Klintmalm et al., 1981; Morris et al., 1983; Chapman et al., 1985)*. A daily dose of 10 mg/kg represents the therapeutic dose of CsA in rats, and gives a comparable blood levels to humans *(Fehman et al., 1987)*. This dose resulted, in the present study, in changes seen in the PCT of juxtamedullary cortex, which ranged from mild attenuation of the epithelial lining, vacuolation of the cytoplasm, to frank ballooning of the cells with pyknotic nuclei. The changes were sporadic and minimal in rats receiving 10 mg/kg CsA and were widespread in rats receiving the toxic dose of 50 mg/kg CsA. similar results of tubular vacuolation were described in experimental animals *(Simons et al., 1980; Whiting et al., 1983; Creagh et al., 1991)* and humans *(Mihatsch et al., 1983)* treated with CsA. Tubular vacuolation was rarely seen by *D'Ardenne et al., (1986)*, which added that it could be caused by the method of tissue preparation. The occasional tubular casts seen in the present work were also described by *Creagh et al., (1991)* in the kidneys of the dogs treated with CsA 10 mg/kg/day for 10 days. Several authors have described tubular necrosis and microcalcification as a result of CsA nephrotoxicity *(Calne, 1980; Fernando et al., 1980; Keown et al., 1981; Schulman et al., 1981; Mihatsch et al., 1983; Myers et al., 1988)*. *Sibley et al., (1983)*, and *Farnsworth et al., (1984)* found these features to be poor discriminators between CsA nephrotoxicity and immunorejection. We could not detect, in the present work, any criteria of tubular necrosis even in rats receiving 50 mg/kg/day CsA for 3 weeks.

The mild mononuclear cellular infiltrates which were occasionally seen in the present work, were similar to those described by several authors. *Farnsworth et al., (1984)* in man, and *Jackson et al., (1987)*, *Gillum et al., (1990)*, *Burdmann et al., (1994)* in rats, have reported similar cellular inflammatory reaction in the kidney after treatment with therapeutic dose of CsA. This interstitial inflammatory infiltrate was claimed to have preceded interstitial fibrosis, an assumption which is supported by the finding that CsA could induce fibroblast proliferation and collagen synthesis in vitro *(Wolf et al., 1990)*. Other authors *(Sibley et al, 1983; D'Ardenne et al., 1986; Neild et al., 1986)* could not attribute the presence of cellular infiltrate to CsA nephrotoxicity; as it was also found during periods of stable kidney function. *D'Ardenne et al., (1986)* attributed interstitial fibrosis to other factors such as chronic rejection, or cardiovascular disease which render the patient more susceptible to the effects of CsA. The present study did not show any interstitial fibrosis; probably the experimental period was too short to elucidate it.

Several authors attributed the pathological effects of CsA to its vascular and glomerular lesions, as arteriolar hyalinosis *(Mihatsch et al., 1983; Thiel et al., 1983; Neild et al., 1986; Myers et al., 1988)*, arteriolar necrosis, and mucoid intimal thickening *(Mihatsch et al., 1983)*, glomerular capillary thrombi *(Shulman et al., 1981; Neild et al., 1985)*, glomerular inflammatory infiltrate and crenation of the basement membrane *(Farnsworth et al., 1984)*. The vascular detrimental effect of CsA was further proved indirectly by the finding that it greatly enhances the vascular injury that occurs in experimental acute serum sickness in

rabbits *(Neild et al., 1983)* and accelerates arteriosclerosis in the spontaneously hypertensive rat *(Ryffel et al., 1985)*. *Farnsworth et al., (1984)*, and *D'Ardenne et al., (1986)* could not find any evidence of CsA induced glomerular capillary thrombi. The present work did not show any apparent histological changes in the renal glomeruli or blood vessels of the CsA-treated rats. Morphometric study, however, showed a statistically significant decrease in the mean diameter of the glomeruli 1, 2 and 3 weeks after treatment with 10 mg CsA ($P < 0.001$), whereas those of the rats treated with 50 mg CsA returned nearly to their normal size at 3 weeks post-treatment; probably as a compensatory hypertrophy of the glomeruli of the large population sizes. We could not find, in the available literature, any morphometric study on the glomeruli of the kidney treated with CsA. The reduction in glomerular size following treatment with CsA could be explained by vasoconstriction of the glomerular capillary loops, since the reduction occurred in all the size classes of the glomeruli. This could be supported by the findings of the previous authors that CsA induces direct decrease in renal blood flow *(Sullivan et al., 1985)*, sustained afferent arteriolar vasoconstriction *(English et al., 1987)*, and significant increase in juxtaglomerular renin *(Burdman et al., 1994)*. Interstitial fibrosis following CsA therapy was considered as a consequence of CsA induced renal vasoconstriction *(Myers et al., 1988; Rosen et al., 1990)*. This was further proved by the ability of chronic infusion of angiotensin II in rats to produce considerable renal interstitial fibrosis *(Johnson et al., 1992)*, and of angiotensin II blocker losartan to prevent the development of interstitial fibrosis *(Burdman et al., 1994)*.

It is concluded that the most prominent histological picture of CsA nephrotoxicity in the rat is vacuolation of the cells of the PCT. The decrease in the size of the glomeruli without any apparent pathological changes is supporting the view that constriction of the glomerular capillary loops result from CsA nephrotoxicity. It is possible that the vasoconstriction may have lead to ischaemic changes, which are manifested by tubular vacuolation and morphometric glomerular reduction in size.

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تأثير (السيكلوسبورين – أ) على التركيب النسيجي لكلية الفأر

مصطفى النجار، أحمد العياط

(من قسم التشريح – كلية الطب والعلوم الطبية – جامعة الملك عبد العزيز – جدة – المملكة العربية السعودية)

لقد أثبت «السيكلوسبورين – أ» كفاءته كمثبط مناعي قوي مما جعله العقار المختار في عمليات زراعة الأنسجة. وتبع استخدامه تجريبيا وعلاجيا حدوث تسمم كلوي كمضاعفات رئيسية. وبالرغم من تأثيره الثابت على الكلية فإن التغيرات الشكلية السمية على الكلية التي سجلت كانت متضاربة وغير محددة. لذلك تقرر عمل هذه الدراسة لفحص التغيرات التركيبية للكلية في الفئران المعالجة بعقار «السيكلوسبورين – أ».

تم حقن مجموعتان من الفئران بعقار «السيكلوسبورين – أ» الأولى بجرعة ١٠ مجم/كجم في اليوم، والثانية بجرعة ٥٠ مجم/كجم في اليوم. وتم حقن مجموعة ثالثة بنفس الكمية من الذيب مع محلول ملحي فقط واستخدمت كمجموعة ضابطة. وأخذت العينات من الكلى ومررت لعمل شرائح بارافينية بعد ١، ٢ و٣ أسابيع من بداية الحقن. وتم صباغة الشرائح «بالهيماتوكسلين والأيوسين» و«البير أيودك أسد تشيف» وكذلك بصبغة «فان جيسون». وقد لوحظ وجود تغيرات نسيجية متفرقة في الأنابيب القريبة الملتوية خاصة الموجودة في المنطقة ما بين القشرة واللب. وخلايا هذه الأنابيب كانت منتفخة مع وجود أماكن بها فراغات في السيتوبلازم وذلك بعد أسبوع من حقنها بجرعة ١٠ مجم/كجم في اليوم. وقد شوهدت هذه الفراغات في عدد أكثر من الأنابيب بعد ٢ و٣ أسابيع من الحقن مع وجود بعض مناطق بها زيادة خلوية غالبيتها خلايا ليمفاوية وماكروفاج. وقد لوحظ تقلطح الخلايا في بعض الأنابيب مع اتساع في قنواتها وذلك بعد ٣ أسابيع من الحقن. ولقد شوهدت تغيرات معاثلة في كلى الفئران التي عولجت بجرعة ٥٠ ملجم/كجم في اليوم. كما أن هذه التغيرات كانت واضحة وأثرت على مساحة أكبر بعد ٣ أسابيع حيث أظهرت خلايا الأنابيب أماكن مفرغة كبيرة وكانت النوايا داكنة ومنكمشة.

لم تظهر كبيبات الكلية أي تغيرات نسيجية واضحة في الفئران المعالجة «بالسيكلوسبورين – أ». ومع ذلك فقد وجد بالتحليل القياسي أن هناك نقصان ذو أهمية إحصائية في متوسط محيط كبيبات الكلية بعد أسبوع من الحقن بجرعة ١٠ ملجم/كجم في اليوم تلاه إزدياد بسيط بعد ٢ و٣ أسابيع ومع ذلك ظل محيط الكبيبات أقل إحصائيا بمقارنته بالمجموعة الضابطة. وقد لوحظ تغيرات في محيط الكبيبات في الفئران المعالجة بجرعة ٥٠ ملجم/كجم في اليوم وعلى الرغم من ذلك فقد

عادت إلى حجمها الطبيعي بعد ٣ أسابيع من الحقن. وقد وجد أن التوزيع التراكمي التكراري لمحيط الكبيبات اظهر أن تعدادها يحتوى على مدى من الأحجام. وقد اظهر التوزيع الحجمي التكراري لمحيط الكبيبات أن الحجم الغالب في المجموعة الضابطة يتراوح من ١٢٠ إلى ١٣٥ ميكرون و١٠٥ – ١٢٠ ميكرون في الفئران المعالجة بجرعة ١٠ ملجم/كجم في اليوم بعد أسبوع بينما بعد ٢ و٣ أسابيع كانت ١٢٠ – ١٣٥ ميكرون. وقد اظهرت الكبيبات في الفئران المعالجة بجرعة ٥٠ ملجم/كجم في اليوم أن غالبيتها يقع محيطها بين ١٢٠ إلى ١٣٠ ميكرون بعد أسبوع ثم نقصت إلى ١٠٥ – ١٢٠ ميكرون بعد أسبوعين ثم زادت إلى ١٣٥ – ١٥٠ ميكرون بعد ٣ أسابيع. وهذا النقصان في حجم الكبيبات دون وجود أى تغيرات مرضية ظاهرة بها يعنى انه من المحتمل وجود ضيق في الأوعية الدموية المغذية للكبيبات. والنتائج القياسية للكبيبات تدعم رؤية أن التأثير المرضي «للسيكلوسبورين – أ» على السمية الكلوية نشأت من خلال تأثيره على التغذية الدموية للكبيبات.

(٢١/٢٣١)

after 3 weeks post-treatment. The cumulative frequency distribution of the profile diameters of the glomeruli showed that their population contain a range of sizes. The size-frequency distribution of the glomerular diameters showed that their predominant size-class in the control rats lies at diameters of 120-135 μm. Rats treated with 10 mg CsA showed predominant size-class glomeruli at lower diameters of 105-120 μm after 1 week, whereas at 2 and 3 weeks at predominant glomeruli lied at diameters of 120-135 μm. Rats treated with 50 mg CsA showed predominant glomeruli at diameters of 120-135 μm after 1 week, decreased to 105-120 μm at 2 weeks, and increased to 135-150 μm at 3 weeks. The reduction of the size of the glomeruli, without apparent pathological changes probably denotes vasoconstriction of the glomerular tufts. The glomerular morphometric findings are supporting the view that the pathogenic effect of CsA nephrotoxicity could be mediated through its vasoconstrictor effect on the glomerular vasculature.

INTRODUCTION

Since the introduction of clinical renal transplantation by Murray, Merrill and Harrison in 1954 perhaps the most exciting advance in transplantation has been the discovery of cyclosporin *(Borel, 1976; Brower et al., 1977)*. Its efficiency as an immunosuppressive agent have made it the drug of choice in transplantation of kidney, heart and liver *(Calne et al., 1978; Kahan, 1985)*. However, nephrotoxicity was reported as a major complication of cyclosporin (CsA) therapy in patients of bone marrow *(Powles et al., 1980; Gluckman et al., 1981)*, liver *(Klintmalm et al., 1981)*, and renal grafts *(Calne et al., 1978 and 1979)*.

Various renal histological abnormalities have been ascribed to CsA, including vacuolation of proximal tubular epithelial cells *(Simons et al., 1980; Mihatsch et al., 1983; Creagh et al., 1991)*, tubular epithelial cell microcalcification *(Mihatsch et al., 1983)*, arteriolar hyalinosis and mucoid intimal thickening *(Mihatsch et al., 1983; Neild et al., 1986; Myers et al., 1988)*, glomerular capillary and arteriolar thrombosis *(Shulman et al., 1981; Neild et al., 1985)*, mononuclear cell infiltrates *(Sibley et al., 1983; Jackson et al., 1987; Gillum et al., 1990; Burdman et al., 1994)*, and diffuse interstitial fibrosis *(Farnsworth et al., 1984; Klintmalm et al., 1984; Myers et al., 1988)*. Other authors did not find a proof that CsA treatment is the cause for occurrence of epithelial tubular vacuolation *(Farnsworth et al., 1984; D'Ardenne et al., 1986)*, glomerular capillary thrombi, *(Farnsworth et al., 1984; D'Ardenne et al., 1986)*, interstitial fibrosis *(D'Ardenne et al., 1986)*, focal mononuclear cell infiltrates *(Neild et al., 1986)*. The morphological criteria described in CsA nephrotoxicity were mostly obtained from patients or animals receiving transplanted organs. This may render other factors as immunorejection, traumatic or vascular ischaemia play a major role as a cause for the morphological picture, making the histological changes that have been reported largely nonspecific. Proper documentation of the morphological appearance of CsA nephrotoxicity is valuable for distinction between it and renal rejection when used in renal transplantation; since the management and outcome of these two complications are different. The present study was,

therefore, undertaken to examine the effect of cyclosporin on the histological structure of the rat kidney.

MATERIALS and METHODS

Animals :

Forty-five adult male Wistar rats weighing 220-250 g were used in this study. They were obtained from the Animal House of King Fahd Medical Research Center, King Abdulaziz University, Jeddah, Saudi Arabia. The rats were kept in a controlled environment (constant temperature 24°C, and a 12 h light-dark cycle), and were maintained on a standard diet and water *ad libitum*.

Treatment with CsA :

The rat were divided into three groups, 15 animals each. The first two groups were injected subcutaneously with a daily dose of 10 and 50 mg/kg body weight of CsA, respectively. Cyclosporin A concentrate (Sandimmun, from Sandoz, Basle, Switzerland), containing 50 mg/ml cyclosporin in 650 mg polyoxyethylated castor oil and 33% alcohol as vehicle, was diluted to 2 and 10 mg/ml with normal milipore filtered saline and used for subcutaneous injection. Pharmacokinetic studies indicated that subcutaneous route allows steady and reproducible plasma levels of CsA to be obtained, with only very little variation, over the 24 h following administration *(Wassef et al., 1983)*. The third group was injected daily with a similar volume of the vehicle with saline alone, and used as a control. The rats were housed in individual cages and allowed free access to food and water.

Histological examination :

Five rats from each group were sacrificed 1, 2 and 3 weeks after starting the injection. The rats were anaesthetized with ether and abdominal viscera exposed by a midline incision. The rats were bled to death by cutting the abdominal aorta. The kidneys were quickly removed and fixed in 10% formol saline and were embedded in paraffin. Four μm thick sections were stained with hematoxylin and eosin, periodic acid schiff (PAS), and Van Giesson and examined with light microscope. Histological sections were examined according to the protocol described by *D'Ardenne et al., (1986)*, for the following changes :

1. *Tubular changes:* cytoplasmic vacuolation, flattening or necrosis of epithelial cells, intraluminal casts, and cellular infiltrates.
2. *Glomerular changes:* mesangial expansion, cellular infiltrates and capillary thrombosis.
3. *Interstitial changes:* cellular infiltrates, edema, fibrosis and hemorrhage.
4. *Vascular chagnes:* intimal edema, fibrosis and necrosis.

Morphometric Analysis :

Morphometric study was carried out on renal glomeruli of the hematoxylin and eosin and PAS stained sections. A total of 2660 glomerular profiles were measured throughout the study (382 from control, 400, 406 and 416 glomeruli from the rats treated for 1, 2 and 3 weeks, respectively with 10 mg CsA and 350, 360 and 345 glomeruli from the rats treated for 1, 2 and 3 weeks with 50 mg CsA, respectively). Renal glomerular profiles were chosen at random from each slide and were morphometrically analyzed at a magnification of 400 x. The maximum (a) and minimum (b) individual glomerular diameters between the internal edge of Bowman's capsule were measured by an

ocular micrometer. The profile diameter (d) of each glomerulus was calculated from the equation $d=\sqrt{ab}$. The size-frequency distribution of the profile diameters was plotted. The mean axial ratio of the profiles was calculated. Assuming that the glomeruli are spheroid structures, the formula of Pullman *(Williams, 1977)*, for the ungrouped profile range of sizes, was used to calculate the mean diameter (D) for the glomeruli of each specimen.

$$\bar{D} = \frac{\pi}{2} \times \frac{N}{1/d1 + 1/d2 \ldots\ldots 1/dN}$$

where N represents the total profiles measured and d1, d2, ..., dN represent the profile diameters.

Statistical analysis :

Values were presented as means ± standard error of mean. Data obtained from the groups of rats treated with 10 and 50 mg of CsA were analyzed statistically by one way analysis of variance (ANOVA) followed by LSD test pairwise comparisons using a current SPSS statistical package. Student's t-test was used for statistical comparison of the renal glomeruli of each CsA treated group and the control group. The level of significance was determined to be less than 0.05 throughout the study.

RESULTS

The kidneys of the control rats, which received injection of the vehicle in saline for 3 weeks, showed normal appearance of renal tubules (Fig. 1). One week after injection with CsA 10 mg/kg/day, the kidneys showed minimal changes in few proximal convoluted tubules (PCT) which were arranged in groups, mostly located at the juxtamedullary cortex. The lining cells of these tubules appeared slightly swollen and contained peripheral nuclei and lightly eosinophilic cytoplasm which showed small and medium-sized vacuolar spaces (Fig. 2). Their cytoplasm stained weakly positive with PAS, and the tubules showed well defined basement membrane and variably defined luminal border. Similar findings were seen 2 weeks after injection of CsA 10 mg/kg/day (Fig. 3), the changes, however, could be seen affecting greater number of PCTs. The specimens examined 3 weeks after CsA showed, in addition to the above mentioned changes, flattening of cells in some of the PCTs with widening of their lumen and increased cellularity of the interstitial tissue around them (Fig. 4). Occasional tubules with intraluminal casts were rarely seen.

One week after injection with CsA 50 mg/kg/day, the kidneys showed changes in an area of PCT which was apparently larger than that seen with 10 mg CsA. The affected tubules were found in groups, mostly located at the juxtamedullary cortex, although some areas of affected tubules could be noted beneath the kidney capsule (Fig. 5). The cells of the affected tubules contained lightly-stained cytoplasm with vacuolar spaces, and peripheral nuclei. Two weeks after injection of CsA 50 mg/kg/day, a larger area of tubules was shown to be affected (Fig. 6). The specimens examined 3 weeks after 50 mg of CsA showed focal changes affecting groups of tubules (Fig. 7). The cells were lightly stained, and showed ballooning of their cytoplasm with vacuolar spaces and dark pyknotic nuclei. Flattening of cells in some of the tubules with widening of their lumen could be seen. Occasional cells, mostly

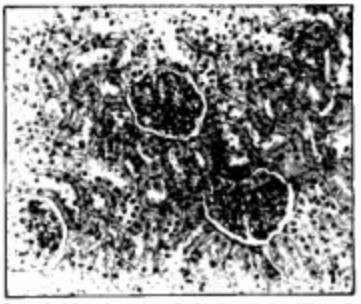

Fig. 1: Cortex of a kidney of control rat, after injection with the vehicle in saline for 3 weeks, showing renal tubules and glomeruli of normal appearance.
(PAS X 224)

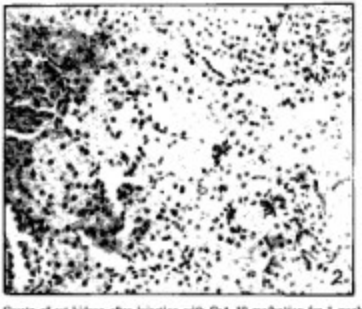

Fig. 2: Cortex of rat kidney after injection with CsA 10 mg/kg/day for 1 week. Few tubules show cells with slightly swollen cytoplasm and small vacuolar spaces.
(Hz. & E. X 224)

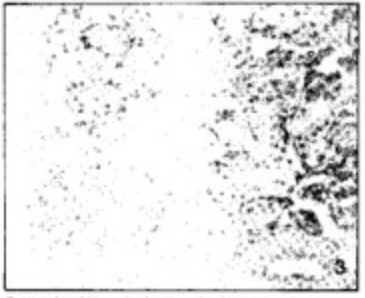

Fig. 3: Cortex of rat kidney after injection with CsA 10 mg/kg/day for 2 weeks. A group of tubules shows cells containing vacuolar cytoplasm.
(PAS X 224)

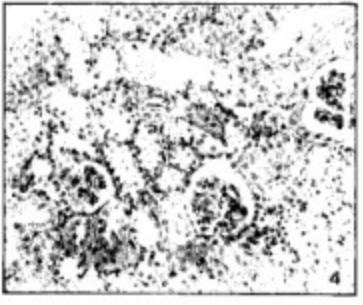

Fig. 4: Rat kidney after injection with CsA 10 mg/kg/day for 3 weeks. Some tubules are showing cells with vacuolar cytoplasm, and few tubules shows flattening of their epithelial lining. (PASX 224)

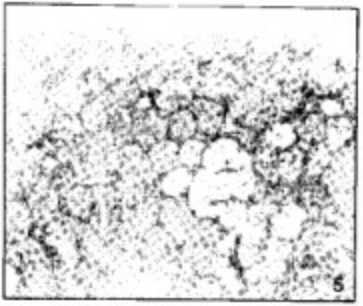

Fig. 5: Cortex of rat kidney after injection with CsA 50 mg/kg/day for 1 week. Few tubules are showing swollen cells with vacuolar cytoplasm. Subcapsular tubules with vacuolated cytoplasm could also be seen. *(H&E X 224)*

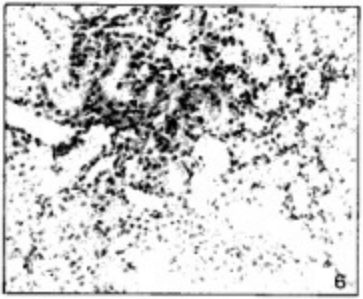

Fig. 6: Juxtamedullary cortex of rat kidney after injection with CsA 50 mg/kg/day for 2 weeks. Group of tubules are showing swollen cells with vacuolar cytoplasm. *(Hx. & E. 224)*

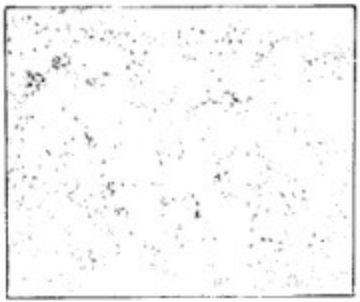

Fig. 7. Juxtamedullary cortex of rat kidney after injection with CsA 50 mg/kg/day for 3 weeks. Large number of tubules are lined with cells showing vacuolar cytoplasm and pyknotic nuclei. *Oil, ob L. X 210.*

lymphocytes and macrophages were found infiltrating the affected areas. Few tubules with intraluminal casts could also be seen. We could not detect, however, any apparent evidence of cellular necrosis among the different cells of the tubules. Slides stained with Van Giesson did not show any sign of interstitial fibrosis.

Morphometric study of the renal glomeruli showed that their profile diameters have mean axial ratio of less than 1.243 in the examined specimens (Table 1), indicating that the glomeruli could be treated as spheroids. The cumulative frequency distribution of the profile diameters of the glomeruli showed that their population contains a range of sizes, justifying the use of the formula of Fullman (*Williams, 1977*) to calculate the mean true diameters (D). Although the renal glomeruli of the CsA-treated rats did not show any apparent histological changes, morphometric data showed that they were quantitatively different from those of the control. The results of ANOVA showed that the diameters of the renal glomeruli of the control group and rats treated with 10 mg/kg/day CsA for 1, 2 and 3 weeks were significantly different from each other. Statistically significant decrease in the diameter of the glomeruli was found 1 week after treatment with 10 mg CsA ($P < 0.001$). This indicates that there had been some loss of the glomerular mass following treatment with 10 mg CsA. Slight recovery of the glomerular diameter was shown with advancing treatment period with CsA, as indicated by an increase in their mean diameter at 2, and 3 weeks post-treatment (Table 1), they were still, however, significantly below that of the control rats ($P < 0.001$). similar decrease in the diameters of the glomeruli of the rats treated with 50 mg CsA was noticed, however, they returned nearly to their normal size after 3 weeks

Table 1: Diameters and axial ratio of the renal glomeruli of rats treated with CsA at doses of 10 and 50 mg/kg/day for 1, 2 and 3 weeks. *

Experimental group	*Number of Animals*	*Diameter*	*Axial Ratio*
Control	15	128.088 ± 1.369	1.221 ± 0.008
10 mg CsA, 1 W	5	111.463 ± 1.066 **	1.190 ± 0.007
10 mg CsA, 2 W	5	117.619 ± 1.176 **	1.197 — 0.008
10 mg CsA, 3 W	5	121.391 ± 1.062 **	1.191 ± 0.007
50 mg CsA, 1 W	5	107.241 ± 1.231 **	1.243 ± 0.009
50 mg CsA, 2 W	5	109.160 ± 1.166 **	1.183 ± 0.008
50 mg CsA, 3 W	5	126.974 ± 1.498	1.173 ± 0.007

* Values are presented as means ± SEM.
** Significant difference between this group and the control at $P < 0.001$.

post-treatment (Table 1). The results of ANOVA showed that the diameters of the renal glomeruli of the rats treated with 50 mg CsA for 1 and 2 weeks were significantly smaller than those of the control rats. No significant difference, however, was found between the diameters of the renal glomeruli of the rats after 3 weeks treatment with 50 mg CsA and the control, or between the renal glomeruli after 1 and 2 weeks treatment with 50 mg CsA.

The size-frequency distribution of the profile diameters of the glomeruli showed an overall shifting of all the size classes toward smaller sizes, 1 week after 10 mg/day CsA, as compared to the corresponding classes of the glomeruli of the control rats (Fig. 8a). Whereas the predominant glomeruli (27.7%) in the control rats were of 120-135 µm diameters, those of rats treated with 10 mg CsA for 1 week (32.5%) were 105-120 µm in diameter. The predominant population of glomeruli of rats treated with 10 mg CsA/day for 2 and 3 weeks (forming 27.6% and 30.8%, respectively) returned to the size of the normal rats; 120-135 µm (Fig. 8a). The size population of the glomeruli of rats treated with 50 mg CsA showed that the predominant glomeruli (30.8%) lied at diameters of 120-135 µm after 1 week, decreased to 105-120 µm (forming 34.2%) at 2 weeks, and increased to 135-150 µm (forming 26.1%) at 3 weeks of treatment with of CsA (Fig. 8b).

DISCUSSION

Cyclosporin A (CsA) has been known as a very potent immunosuppressive agent. However, the ability of this drug to cause impaired renal function was quickly recognized (*Calne et al., 1978*). Clinical nephrotoxicity was manifested by raised