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Effect of reduced cyclosporin dosage on long-term renal allograft histology

H. E. Wilczek[1], C. G. Groth[1], and Sven-Olof Bohman[2]

[1] Department of Transplantation Surgery and [2] Department of Pathology, Huddinge Hospital and Laboratory for Biological Structure Research, Department of Anatomy, Karolinska Institute, S-14186 Huddinge, Sweden

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Abstract. The effect of different doses of cyclosporin (CyA) on the occurrence of histological lesions in renal allograft biopsies was investigated 2 years after transplantation. Biopsy findings were compared in three different groups of patients. In group 1, patients were immunosuppressed with CyA and prednisolone according to an early, high-dosage schedule (initial CyA dose 15–17.5 g/kg body weight); in group 2, they were treated with a medium CyA dose (initial dose 12 mg/kg), together with prednisolone; and in group 3, patients were given triple drug therapy consisting of low doses of CyA (initial dose 8 mg/kg), together with both azathioprine and prednisolone. Interstitial fibrosis and tubular atrophy were common findings in all groups, and on the basis of all biopsies, no difference could be found between the groups with respect to the relative volume of the renal cortical interstitium, which was used as a quantitative parameter for interstitial fibrosis. Likewise, no difference was found with respect to serum creatinine levels. When grafts that showed signs of rejection (usually vascular rejection) in the biopsy were excluded (two in group 1, six in group 2, and ten in group 3), the mean interstitial volume was significantly lower in group 3 (triple drug therapy) than in the other groups. The serum creatinine levels were also significantly lower in group 3 than in group 1. Thus, chronic renal lesions could be ameliorated when CyA doses were lowered, but this appeared to entail an increased risk of acute or chronic vascular rejection.

Key words: Cyclosporin, kidney histology – Kidney transplantation, cyclosporin, histology – Histology in kidney transplantation, cyclosporin – Cyclosporin, reduced dose, kidney histology

Chronic renal damage induced by cyclosporin A (CyA) was first described in recipients of kidney [13, 35] and heart [20] transplants. It was later confirmed in studies of patients treated with CyA for autoimmune diseases [28, 32, 33], as well as in recent studies of animal models [2, 30, 31]. Against this background, two questions with important clinical implications arise: (1) Do the described chronic renal lesions progress in patients treated with maintenance doses of CyA? and (2) Can chronic renal damage be avoided or reduced by using a lower CyA dose? In a previous paper [39], we found no clear-cut evidence for the progression of interstitial fibrosis in renal grafts followed by repeated biopsies up to 5 years after transplantation. The results of clinical follow-up studies [17] are compatible with this finding, although highly insensitive parameters of renal function, such as the serum creatinine level, have usually been used. Furthermore, in CyA-treated heart transplant recipients, a more unfavorable course has been reported [22]. The reasons for these discrepancies have not been fully clarified.

In this study, we investigated the possible influence of lowering the CyA dose on the occurrence of histological lesions in renal allograft biopsies obtained 2 years after transplantation. We compared the biopsy findings in three patient groups: one group immunosuppressed with CyA and prednisolone according to an early, high-dosage schedule in our hospital (initial CyA dose 15–17.5 mg/kg body weight), a second group treated with lower doses of CyA (initial dose 12 mg/kg body weight), together with prednisolone, and a third group given triple therapy consisting of low doses of CyA (initial dose 8 mg/kg body weight), together with both azathioprine and prednisolone [16]. Fewer cases of chronic CyA toxicity occurred in the latter group, but there was instead an increased frequency of vascular rejection.

Materials and methods

Patients

All of the patients in our study received kidney transplants at the Department of Transplantation Surgery of Huddinge Hospital. These patients belonged to three different groups with respect to immuno-

Offprint requests to: H. E. Wilczek

Table 1. Patients belonging to three different cyclosporin (CyA) dosage protocols and investigated with renal allograft biopsy. All values represent mean ± standard deviation

	Group 1 (High-dose CyA protocol[a]) $n = 25$	Group 2 (Medium-dose CyA) $n = 35$	Group 3 (Triple drug therapy) $n = 34$
Initial CyA dose (mg/kg body weight per day)	15–17.5	12	8
Recipient age (years)	44 ± 15	42 ± 15	46 ± 13
Donor age (years)	42 ± 13	43 ± 18	52 ± 13
No. of HLA-AB incompatibilities (mean)	2.1 ± 1.2	2.0 ± 1.0	2.2 ± 1.2
No. of HLA-DR incompatibilities (mean)	0.6 ± 0.7	1.1 ± 0.7	1.2 ± 0.7
Time interval between transplantation and biopsy (months)	24 ± 3	25 ± 3	25 ± 2
Serum CyA concentration at the time of biopsy (ng/ml)	401 ± 283	386 ± 207	320 ± 200
CyA dose at the time of biopsy (mg/kg body weight per day)	5.9 ± 2.9	4.1 ± 1.9	3.4 ± 1.3
Cumulative CyA dose (g)			
at 1 month	30 ± 15	20 ± 5	14 ± 3
at 3 months	72 ± 22	47 ± 10	36 ± 10
at 6 months	122 ± 34	80 ± 17	63 ± 19
at the time of biopsy	359 ± 118	262 ± 63	206 ± 15
No. of antirejection treatments prior to biopsy (mean)	0.7 ± 0.7	0.9 ± 1.1	1.1 ± 1.0

[a] Patients were immunosuppressed with CyA and prednisolone according to the protocol of the First Scandinavian Multicenter Study [15]

suppression: group 1 (high-dose CyA protocol) consisted of patients who underwent transplantation in 1981–1985 and who received CyA in an initial dose of 17.5 or 15 mg/kg body weight, together with prednisolone (according to the dosage schedule of the First Scandinavian Multicenter Study [15]); group 2 (medium CyA dose protocol) consisted of 35 patients transplanted in 1985–1987 who received a low dose of CyA (initial dose 12 mg/kg), together with prednisolone; group 3 (triple drug therapy) consisted of 34 patients transplanted in 1985–1987 who received immunosuppression with CyA (initial dose 8 mg/kg), together with azathioprine (Aza) and prednisolone. During the period 1985–1987, patients were randomly assigned to either group 2 or 3 (Second Scandinavian Multicenter Study [16]). Further details concerning the patients and their immunosuppressive treatment are presented in Table 1.

There was no difference between the groups with respect to the age of the recipients, but donor age was slightly higher in the triple drug group (group 3) than in the other groups. Both the CyA dose at the time of biopsy and the cumulative CyA dose at various points in time differed significantly between the groups (Table 1). The patients in the triple drug group (group 3) had slightly more acute rejection episodes than those in group 1, but the difference was not statistically significant.

In order to make comparisons, 32 renal allograft biopsies obtained from patients immunosuppressed with Aza and prednisolone were also analyzed. These biopsies were taken from patients who had undergone transplantation between 1972 and 1982; the interval between transplantation and biopsy was 3–13 years (mean 7.25 years), which was considerably longer than in the three CyA-treated groups (Table 1).

Biopsy technique

All biopsies were obtained according to a planned protocol for clinical and research follow-up at the Department of Transplantation Surgery of Huddinge Hospital. They were taken as close as practically possible to the 2-year day after transplantation, irrespective of renal transplant function at the time of biopsy. All of the patients had given their informed consent and the study was approved by the Committee for Medical Ethics at Huddinge Hospital.

The percutaneous core needle biopsy method was used as described elsewhere [38]. The location of the kidney graft was determined with the aid of the operation report and manual palpation, and the biopsy was obtained under local anesthesia, using a Tru-Cut disposable biopsy needle (outer diameter 2.0 mm, Travenol Labs, Deerfield, Ill., USA). After biopsy, firm manual pressure was applied to the site of puncture for 10 min, and thereafter the patient had to remain in bed for a minimum of 4 h. The patient was allowed to leave the hospital on the day of biopsy, after voiding urine.

Biopsy processing and analysis

The biopsies were fixed in 3% buffered formalin and embedded in paraffin according to routine procedures. Sections were cut at 3 μm and stained with hematoxylin-eosin, Ladewig's trichrome stain, and silver methenamine.

Microscopy of the biopsies was carried out without knowledge of the group to which the patient belonged. The relative volume (volume density, V_V) of the renal cortical interstitium was used as a parameter for renal interstitial fibrosis with tubular atrophy, which is the usual finding in chronic CyA nephrotoxicity [13, 35]. For this purpose, the areal density (A_A, relative section area) of the cortical interstitium was determined by point counting [37], using a 5 × 5 point grid (Integralplatte I, Zeiss Sweden, Stockholm, Sweden) that was inserted in the 10 × eyepiece of a Zeiss standard light microscope equipped with a plan 40 × objective lens. The volume density of the interstitium was calculated according to the formula $P_P = A_A = V_V$ [37]. In addition to this quantitative analysis, the following histological changes were semiquantitatively assessed on a 0–4 + scale: interstitial inflammation, arteriolar hyalinosis, arteriolar smooth muscle degeneration, arteriolar intimal swelling and thrombosis, arterial intimal fibrosis, arterial signs of chronic vascular rejection (intimal cell proliferation, intimal foam cells, myxoid degeneration), and arterial signs of acute vascular rejection (endarteritis, vascular wall necrosis, thrombosis). For some purposes, the sum of scores for the various arteriolar lesions (range 0–12 +) was used as a measure of arteriolo-

Table 2. Renal allograft function and biopsy findings in patients treated with different doses of cyclosporin (CyA). All values represent mean ± standard deviation

	Group 1 (High-dose CyA protocol[a]) $n = 25$	Group 2 (Medium-dose CyA) $n = 35$	Group 3 (Triple drug therapy) $n = 34$
Serum creatinine level at the time of biopsy (μmol/l)	211 ± 80	191 ± 87	187 ± 90
V_V (volume density, relative volume) of the renal cortical interstitium (%)	38 ± 12	39 ± 10	35 ± 9
Arteriolar changes (mean of sum of semiquantitative scores)	1.6 ± 2.1	1.7 ± 1.9	1.3 ± 1.9

[a] Patients were immunosuppressed with CyA and prednisolone according to the protocol of the First Scandinavian Multicenter Study [15]

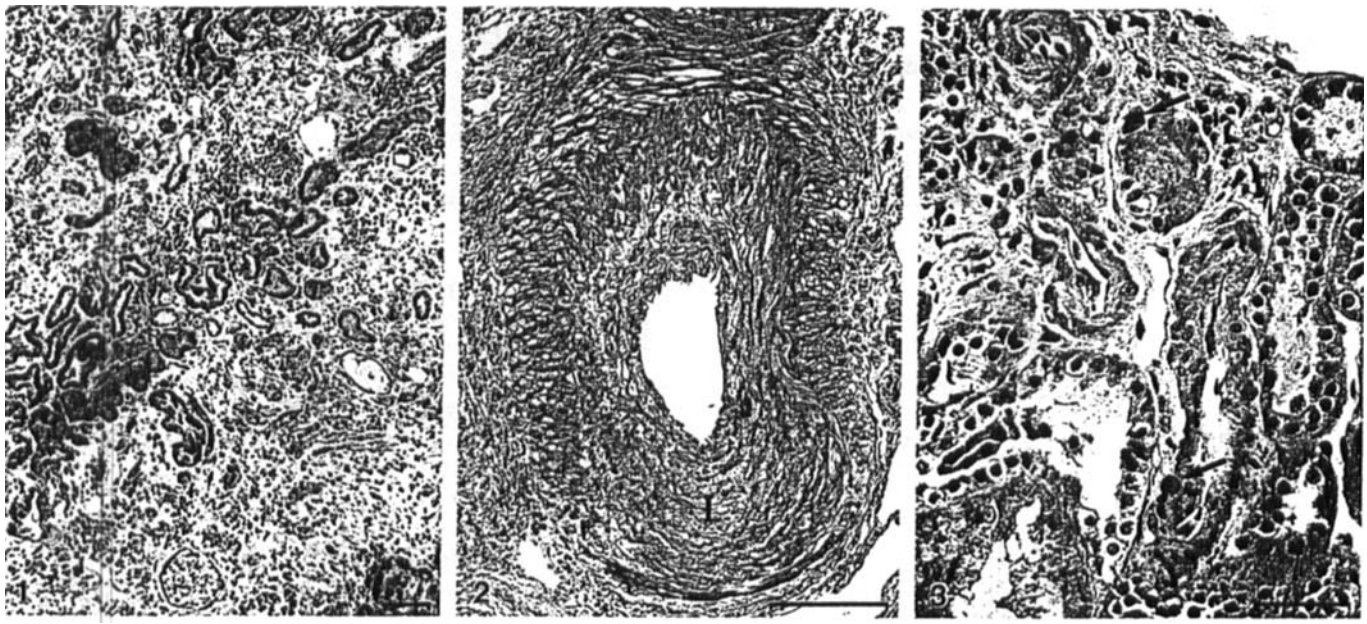

Fig. 1. Biopsy from renal allografts in group 3 affected with severe rejection at 2 years after transplantation. Note the very pronounced interstitial edema and the interstitial inflammatory infiltrate. *Bar:* 100 μm

Fig. 2. Arcuate artery in renal allograft from group 3 with chronic vascular rejection. There is narrowing of the lumen because of considerable thickening of the intima *(I)*. *Bar:* 100 μm

Fig. 3. Arterioles in renal allograft from group 1 showing an irregular thickening of the wall that has a partly destroyed smooth muscle layer and exudation of hyaline material *(arrows)*. *Bar:* 50 μm

pathy. The individual scores for acute and chronic signs of vascular rejection were also sometimes combined (range 0–8 +). The occurrence of glomerular changes and of significant interstitial edema was also recorded.

Statistical analysis

Data were analyzed using linear regression and one-way analysis of variance (ANOVA). For the difference between mean values, Student's *t*-test was used. A *P* value less than 0.05 was considered to indicate a significant difference.

Results

The results with respect to renal allograft function and the relative volume of the cortical interstitium in the grafts were analyzed in two ways. First, an analysis was performed on the whole biopsy material, divided into the different treatment groups described above. Thereafter, a new analysis was performed after exclusion of those biopsies in each group that showed clear-cut signs of acute of chronic rejection.

In the whole biopsy material, no significant differences were found between the CyA groups with respect to the serum creatinine level at the time of biopsy (Table 2). However, all CyA groups had a higher level than that in the azathioprine group (161 ± 90 μmol/l). Likewise, no statistically significant difference between the groups was found regarding the relative volume of the renal cortical interstitium (Table 2). The mean interstitial volume was lower in the azathioprine-treated patients (33% ± 12%) but the difference was significant only at the 10% level. The occurrence of arteriolar lesions did not differ between the CyA groups or between these and the azathioprine group, as judged by the sum of semiquantitative scores for the various types of arteriolar changes (Table 2). The sum of scores in the azathioprine group was 1.4 ± 1.9.

In order to minimize the influence of acute or chronic rejection on the mean interstitial volume density in each group, a new analysis was performed after the exclusion of such biopsies. All biopsies showing either severe interstitial inflammation and/or edema (Fig. 1) or having a sum of semiquantitative scores for acute and vascular rejection of ≥ 2 were excluded before recalculating the parameters mentioned above. It was found that two biopsies in group 1, six biopsies in group 2, and ten biopsies in group 3 showed clear-cut signs of rejection and, therefore, met the exclusion criteria. Thus, significantly more biopsies in group 3 belonged to this category. In most cases, the changes were those of vascular rejection (eight biopsies) with acute and/or chronic lesions (Fig. 2).

After the exclusion of biopsies showing rejection, a significantly lower serum creatinine level was found in group 3 (triple drug therapy), while the values in the other two groups did not differ from those in the previous analysis (Table 3). The mean serum creatinine level in group 3 (165 ± 69 μmol/l) then became similar to that in the azathioprine group (161 ± 90 μmol/l). Moreover, the mean volume density of the renal cortical interstitium was lower in the triple drug group (group 3) than in the other two CyA-treated groups. Also with respect to this parameter, the triple drug group had a value similar to that in the azathioprine group (33% ± 12%). There was a very slight re-

Table 3. Renal allograft function and biopsy findings in cyclosporin (CyA)-treated patients after exclusion of grafts with biopsy signs of rejection. All values represent mean ± standard deviation

	Group 1 (High-dose CyA protocol[a]) $n = 23$	Group 2 (Medium-dose CyA) $n = 29$	Group 3 (Triple drug therapy) $n = 24$
Serum creatinine level at the time of biopsy (μmol/l)	208 ± 80	188 ± 89	165 ± 69[b]
V_V (volume density, relative volume) of the renal cortical interstitium (%)	38 ± 12	38 ± 9	32 ± 8[c]
Arteriolar changes (mean of sum of semiquantitative scores)	1.6 ± 2.1	1.8 ± 1.8	1.1 ± 1.7

[a] Patients immunosuppressed with CyA and prednisolone according to the protocol of the First Scandinavian Multicenter Study [15]
[b] Significantly different from value in group 1 ($P < 0.05$)
[c] Significantly different from values in group 1 and group 2 ($P < 0.05$)

duction in the mean sum of scores with respect to arteriolar lesions (Fig. 3) in group 3, yet no statistically significant differences existed between the CyA dosage groups or between these groups and the azathioprine group (Table 3). No correlation was found between the volume density of the renal cortical interstitium and the severity of arteriolar changes.

Discussion

The main findings in this study were that an amelioration of interstitial fibrosis could be attained using a triple drug immunosuppression protocol with low doses of CyA, but that this effect, with respect to renal allograft function and quantitative data for interstitial volume, was obscured by a simultaneous increase in vascular rejection. In this context, it is important to point out that the quantitative parameter that we have investigated – the volume density or relative volume of the renal cortical interstitium – can be increased not only by interstitial fibrosis and atrophy of the tubules but also by interstitial edema and, to some extent, by interstitial inflammatory cell infiltration. Therefore, it may be necessary to distinguish between these effects when analyzing quantitative data on interstitial volume. In this study, the exclusion of grafts with rejection, i.e., showing severe interstitial edema and/or vascular changes, enabled us to explain our data, which, in the first analysis, appeared somewhat puzzling. Naturally, only biopsies with clear-cut histological evidence of interstitial or vascular rejection could be excluded, and it is therefore possible that some degree of rejection may have been present in a few of the remaining biopsies.

In this study, the volume density (V_V, relative volume) of the renal cortical interstitium was used as a parameter for interstitial fibrosis since there is no simple technique that enables one to determine the degree of interstitial fibrosis per se. In fact, this very common lesion in different forms of chronic renal disease does not imply definite collagenization of the renal parenchyma but largely consists, as recently pointed out by Dische et al. [7], of a combination of atrophy of the tubular epithelium, considerable thickening of the tubular basement membranes, and rearrangement of the surrounding stroma. This leads to an increase in the relative volume of the interstitium (including the thickened basement membranes), which constitutes the basis for using the V_V of the interstitium as a parameter of interstitial fibrosis. A closely related parameter – the so-called tubulointerstitial ratio (TIR) – has been used in some studies [4]. This ratio suffers from the same shortcoming as the V_V of the interstitium in that it can be influenced by tubulointerstitial pathological processes other than interstitial fibrosis (interstitial edema, interstitial cell infiltration, etc.). We chose the V_V of the interstitium in order to avoid the additional influence of different degrees of pathological or artificial tubular epithelial swelling, which may occur in routinely processed kidney biopsies [41].

As pointed out by Oberholzer et al. [24], the quantitative evaluation of cortical interstitial volume in a given kidney requires a minimum size of the renal biopsy in order to become statistically reliable. In this study, we set no size limits for accepting a biopsy for analysis since we were not primarily interested in reliable values regarding each individual kidney. Instead, we compared means of volume densities in different groups of patients. This approach yields a relatively high standard deviation of the mean that limits the sensitivity, for example, with respect to correlations between interstitial volume and various clinical and laboratory parameters and when following individual patients with repeated biopsies [39].

The pathogenesis of interstitial fibrosis in chronic CyA toxicity is not completely known. Interstitial nephritis and toxic tubular damage have been proposed but do not seem to be of major importance in the development of the chronic lesions [3, 5, 30, 33, 36]. Ischemia is considered a more likely mechanism [6, 7, 22]. The latter may be due to vasoconstriction [5, 8, 19, 21] but, in addition, Mihatsch et al. [18] have described an arteriolopathy induced by CyA. Although this type of lesion has been confirmed by some studies [1, 21, 34], other studies [4, 7], as well as the present one, have failed to show any clear-cut correlation between CyA treatment and the occurrence of arteriolar lesions. Whether this can be explained by the difficulties in distinguishing the CyA-induced arteriolar lesions [23] from other arteriolar changes or in detecting the lesions because of their focal distribution remains to be clarified. Clearly, interstitial fibrosis and tubular atrophy can be induced by CyA treatment in experimental animals without the occurrence of histologically detectable arteriolar pathology [2, 30, 31].

One of the main purposes of introducing protocols like the triple drug therapy [10, 29] has been to find a therapy with sufficient immunosuppressive efficacy while at the same time avoiding as much as possible the toxic side effects of the individual drugs, especially acute and chronic CyA nephrotoxicity. Recent clinical follow-up studies on the advantages of triple drug therapy are not

in complete agreement. Although this type of regimen has become popular and may promote patient survival [11], results have been presented which, like our own, show a relatively high proportion of patients with poor renal allograft function during triple drug therapy [27]. It is not always clear whether this is due to CyA nephrotoxicity or to rejection, but our data strongly suggest that the latter – and, in particular, chronic vascular rejection – is an important factor. Similarly, in the study by Fries et al. [11], a relatively high incidence of chronic rejection was reported in the triple therapy patients. It appears that the CyA dose given according to the triple drug protocol may be close to a critical level [12] at which the risk of graft loss increases.

In this study it did not seem necessary in all cases to distinguish between acute and chronic vascular rejection since the definition of the latter and the histological criteria distinguishing these two conditions have not been adequately described [26]. The pathogenesis of chronic vascular rejection is not well understood. It is generally considered to be due to an immunological "attack" [25, 40], although alternative mechanisms have been proposed [9]. However, the role of immunosuppression in its prevention is also unclear. Some investigators have expressed a somewhat pessimistic view with respect to the long-term results of kidney transplantation during CyA immunosuppression, especially with regard to the occurrence of chronic rejection and the uncertainty about optimal long-term maintenance treatment [14]. This study shows that it may be of the utmost importance to know which type of pathological process affects the grafts when different immunosuppressive principles are discussed.

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