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Cyclosporine associated lesions in native kidneys of diabetic pancreas transplant recipients

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Cyclosporine associated lesions in native kidneys of diabetic pancreas transplant recipients. Five years of normoglycemia following pancreas transplantation (PT) does not ameliorate glomerular lesions in patients with their own kidneys and with long-term insulin-dependent diabetes (IDDM) (*Lancet* 342:1193, 1993). All these patients received cyclosporine (CsA) as part of their immunosuppression. Here we examined the relationship of CsA dose and blood levels to the presence and severity of CsA-associated renal lesions and changes in renal function in these PT patients. Renal biopsies were taken before (0) and two and five years after PT from 13 non-uremic IDDM patients and were compared with baseline and five year biopsies from 10 IDDM controls (C). CsA dose was reduced from 10 ± 3 mg/kg/day in the first month to 5 ± 2 in the fifth year post-PT. Creatinine clearance (C_{Cr}) decreased by 34% at one year post-PT and was stable thereafter, and did not change in C. The decline in C_{Cr} from 0 to one year was related to CsA blood levels and dose ($P < 0.005$) at one year. Cortical interstitial volume fraction [Vv(Int/Cortex)], the index of tubular atrophy, and % sclerotic glomeruli increased significantly from 0 to five years post-PT ($P < 0.005$, 0.01 and 0.001, respectively), but did not change in C. There was no significant change from 0 to two years post-PT in these lesions, while there was a clear progression from two to five years. Mean CsA dose and blood levels in the first year post-PT correlated with the increase (Δ) in Vv(Int/Cortex) at five years ($P < 0.05$ for both). The best predictor of Δ Vv(Int/Cortex) was the change in C_{Cr} over the first year post-PT ($P < 0.003$). In conclusion, in five years serious tubulointerstitial and glomerulosclerotic lesions developed in PT recipients on CsA therapy, but not in IDDM C. These lesions were best predicted by the decline in C_{Cr} and CsA blood levels and dose during the first year post-PT. Despite early CsA dose reductions, and stabilization of C_{Cr}, structural lesions progressed from two to five years post-PT.

Pancreas transplantation (PT) is being performed with increasing frequency as a cure for insulin-dependent diabetes (IDDM) [1]. We have recently reported that five years of normoglycemia following successful PT is unable to reverse established diabetic glomerular lesions in IDDM patients with their own kidneys [2]. However, the structural abnormalities in the diabetic kidney involve not only glomeruli, but also arterioles, tubules and interstitium [3]. PT recipients currently routinely receive cyclosporine (CsA) as part of their immunosuppressive regimens. This drug is known to have detrimental effects on renal structure including vascular lesions of arteriolar hyalinosis and CsA arteriolopathy, as well as tubular atrophy and striped interstitial fibrosis [4–7]. Most studies on the renal morphologic consequences of CsA therapy have been limited by the lack of kidney biopsies before the institution of CsA, by relatively short duration of CsA therapy, or by the absence of appropriate controls. Several studies have been performed in renal transplant recipients, where it is often impossible to discriminate structural changes consequent to CsA therapy from those of chronic rejection [8, 9]. Also, in some diseases treated with CsA, such as uveitis and psoriasis [10–13], the kidney may be involved, and, again, the lesions of CsA and those of the underlying disease may be present together and may be inseparable. In addition, most studies performed in such patients lacked baseline kidney biopsies, making the understanding of CsA's contribution to the nephropathology difficult to ascertain. Recently, however, Young et al [14] reported progressive tubulointerstitial changes in renal biopsy specimens obtained at one and three years after institution of CsA therapy for severe psoriasis, but found no such changes in untreated psoriasis patients or normal controls.

The present study quantitated renal structural changes consistent with known morphologic consequences of CsA in a group of PT recipients with IDDM. The study compared these PT patients to a group of IDDM patients (comparison group) who did not receive PT or who had a PT which failed early. Both groups had renal biopsies performed at baseline and five years later.

Methods

Patients

The clinical and demographic features of these patients were reported in a study of the effects of PT on diabetic glomerular lesions [2]. Briefly, all patients spent one week in the Clinical Research Center (CRC) at the University of Minnesota for pre-pancreas transplant evaluation, during which they underwent multiple 24-hour urine collections (at least 3) for measurements of creatinine clearance and urinary albumin excretion rate (AER). Blood pressure (BP) was measured repeatedly by the CRC nursing staff. All the patients underwent percutaneous kidney biopsy. Hb A1 was used to assess glycemic control.

Pancreas transplant group

Thirteen IDDM patients [6 males, 7 females; age 32 ± 7 ($\bar{X}$ ± SD) years; duration of IDDM, 20 ± 5 years] were studied before

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and five years after successful PT. After the initial evaluation, the patients underwent segmental or whole organ PT [1]. After induction therapy all patients received triple immunosuppression with prednisone, azathioprine and cyclosporine (CsA) [1]. One patient was on CsA only for the first year following PT; all others were on CsA for the five years of the study. Insulin was discontinued after PT in all 13 patients. Measures of renal function and metabolic parameters were restudied in most patients at 1, 2, 3.5 and 5 years after PT. A renal biopsy was performed in each patient 2.0 ± 0.4 and 5.0 ± 0.3 years after PT. Two of the PT recipients with advanced diabetic nephropathy and severe glomerular and interstitial lesions at baseline underwent kidney transplantation 7.7 and 6.3 years, respectively, after PT.

Comparison group

Ten IDDM patients (1 male, 9 females; age 28 ± 10 years; duration of IDDM, 16 ± 7 years) had renal function studies and kidney biopsy as part of their evaluation for PT. Three received a pancreatic graft that failed within six weeks. The other seven patients either decided against the PT procedure, could not obtain insurance coverage for PT, or had multiple preformed HLA antibodies and a matched graft did not become available. These patients, still diabetic and receiving insulin therapy, volunteered to have renal function studies and kidney biopsies repeated 5.4 ± 1.4 years after the first evaluation. The comparison and the PT patients were similar for age, duration of IDDM, glycemic control, creatinine clearance, mean blood pressure, and AER at baseline [2].

Procedures

These studies were approved by the Committee for the Use of Human Subjects in Research of the University of Minnesota; all patients gave written informed consent before each study. HbA1 was measured by BioRad column assay until November 1986 and by HPLC thereafter (BioRad Diamat, Biorad Laboratories, Hercules, CA, USA). In some of the patients studied in the early 1980s, only total glycosylated hemoglobin was initially measured; therefore in all patients, the values are expressed as total HbA1. Creatinine clearances (C_{Cr}) were measured on urine collections done under careful supervision in our Clinical Research Center. Serum and urine creatinine levels were measured by an automated kinetic method that uses the Jaffe reaction. AER was measured by nephelometry using the Beckman kit (Beckman Instruments, Inc., Fullerton, CA, USA). CsA blood levels were measured by HPLC [15] on blood samples drawn 12 hours after CsA administration.

Renal tissue was obtained by percutaneous biopsy and processed for light and electron microscopy (EM) [16]. Morphometric measurements were performed by a single observer (PF), except for measurements of tubular atrophy and arteriolar hyalinosis (MJM and EHS). Both these observers were unaware of the patients' treatment and identity for these measurements. EM was performed for estimation of glomerular basement membrane (GBM) width and mesangial volume fraction [Vv(mes/glom)]; the results of these EM studies have been previously reported [2].

Tissue for light microscopy was embedded in paraffin, cut in 2 to 3 μm sections and stained with periodic acid-Shiff stain (PAS). The fractional volume of the renal cortex which is interstitium [Vv(Int/Cortex)] was estimated using a projecting microscope at 300× [17]. All available cortical tissue was measured. Points falling on the interstitium, defined as the space outside Bowman's capsule, tubular basement membrane and vessels larger than one tubular diameter, and total number of points overlying the cortical tissue were counted to estimate the Vv(Int/Cortex), using a 1:4 grid with a distance between fine points of 13 mm. The normal values for Vv(Int/cortex) of 0.15 ± 0.02 were determined from biopsies of renal transplant donors [17]. The index of interstitial fibrosis was determined by two observers (MM and PF) without knowledge as to patient identity, with semiquantitative scores applied as follows: 0 = normal; 1+ = early detectable areas of interstitial fibrosis; 2+ = interstitial fibrosis involving 25 to 50% of interstitial areas; 3+ = interstitial fibrosis involving >50% of interstitial areas [18].

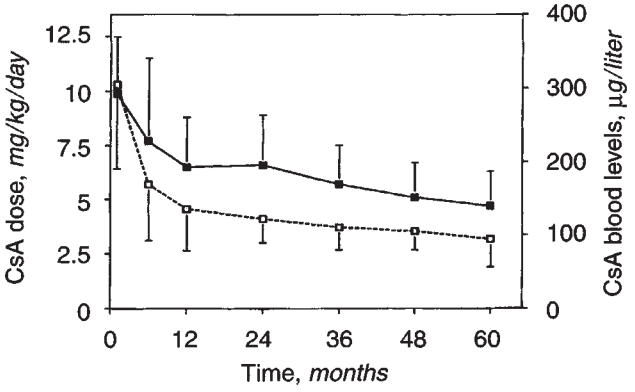

Fig. 1. *CsA mean daily dose (mg/kg/day) (■) and CsA mean blood levels (μg/liter) (□) at 1, 6, 12, 36, 48, and 60 months after PT.* The values represent the means (and 1 SD) of the values for that month. In this and subsequent figures, abbreviations are: CsA, cyclosporine, and PT, pancreas transplantation.

Percent sclerosed glomeruli was determined, as previously described, only when at least 20 glomeruli were available for study [19]. The normal value for percent sclerosed glomeruli is <10% and was derived from biopsies of renal transplant donors [19].

The arteriolar hyalinosis score was obtained by grading as 0 normal arterioles, as 1.0 vessels with <50% of the arteriolar wall replaced by hyaline material and as 2.0 vessels with >50% of the arteriolar wall replaced by hyaline material. The sum of these scores was then divided by the number of the arterioles evaluated.

$$\text{Arteriolar hyalinosis score} = \frac{(1 \times n < 50\%) + (2 \times n > 50\%)}{\text{total number of arterioles}}$$

where n is the number of arterioles.

The index of tubular atrophy is a semiquantitative score going from 0 to 4+: 0 is normal; 1 corresponds to minimal, which means the presence of tubular atrophy in about 1 to 5% of tubules; 2 = slight, corresponding to 5 to 15%; 3 = medium 15 to 30%; and 4 = severe more than 30% [20].

The presence of CsA arteriolopathy was blindly evaluated (by MJM) as previously described [7].

Cyclosporine dose and blood levels

Figure 1 illustrates the changes in mean CsA daily dose and CsA blood levels during the study. Each point in the figure represents the mean value for the corresponding month. At each time a highly significant correlation was observed between CsA daily dose and CsA blood levels ($P < 0.01$ at each time). CsA dose decreased from 9.9 ± 2.6 (10.7, median) in the first month after

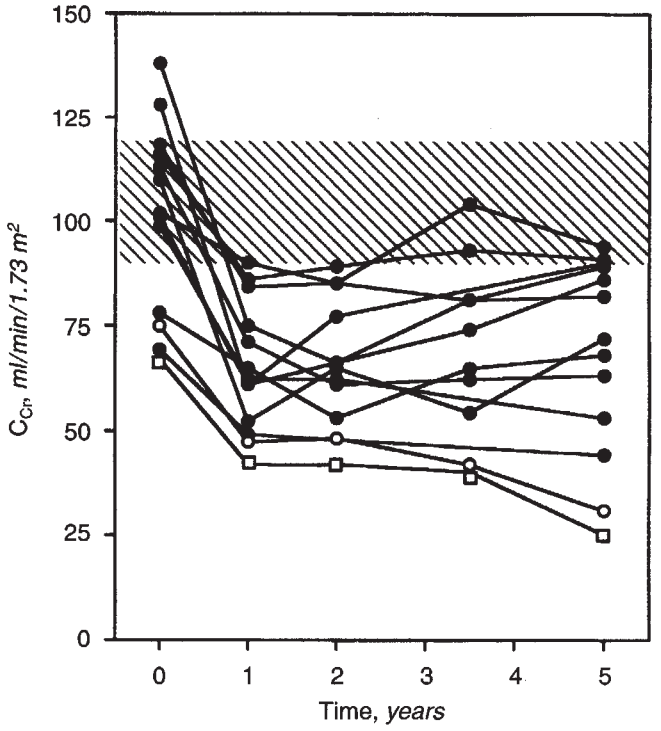

Fig. 2. *Creatinine clearance (C_{Cr}) in recipients of PT at baseline and at 1, 2, 3.5, and 5 years follow-up.* Creatinine clearance fell from 102 ± 21 ml/min/1.73 m^2 to 68 ± 24 ($P < 0.0001$) at five years after PT. The shaded area represents the normal range. In this and subsequent figures, the open symbols refer to the two patients who subsequently required kidney transplantation; □ refers to the patient who was on CsA for only one year.

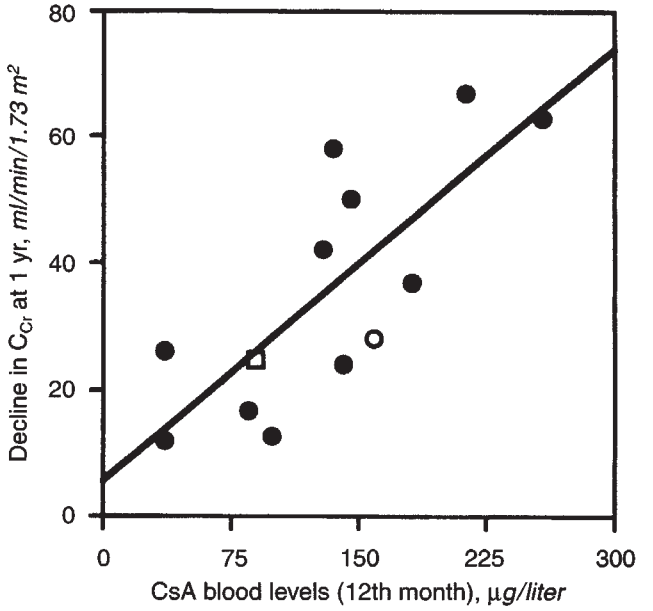

Fig. 3. *Correlation between CsA blood levels during the 12th month post-PT and the magnitude of decline in creatinine clearance (C_{Cr}) from baseline to the one year follow-up visit in the PT patients ($r = 0.75$, $P < 0.004$).*

PT to 4.7 ± 1.6 (3.9) mg/kg/day at five years after PT. CsA blood levels decreased from 305 ± 115 (317) to 94 ± 38 (87) µg/liter.

For statistical analyses cumulative CsA doses and blood levels at different times after PT were used. Thus, the mean daily CsA dose and blood levels, respectively, during the first month (9.9 ± 2.6 mg/kg/day, 305 ± 115 µg/liter), the first three months (10.1 ± 2.4, 265 ± 98), the first six months (9.4 ± 2.7, 227 ± 84), the first 12 months (8.2 ± 2.7, 190 ± 62) after PT, and during the five years of the study (6.1 ± 1.8, 126 ± 33) were used.

Statistics

AER values, since not normally distributed, are expressed as median (range) and were logarithmically transformed before analysis. Results are expressed as $\bar{X}$ ± SD (median). Comparisons of baseline with follow up data used the *t*-test for paired data, while those between the two groups of patients employed the *t*-test for unpaired data. Simple regression analysis was used to evaluate the relationships between CsA dose and plasma levels at different times and functional and structural changes over time. Statistical significance was set at the level of $P < 0.05$. For clarity of presentation all *P* values <0.10 are provided.

Since several parameters were expected to co-correlate, multiple regression models were fit to the data using a stepwise model selection procedure, to determine which parameter correlated more strongly with the dependent variable. The level of significance for a variable to enter the model was set at 0.15.

The following independent variables were considered in each of the analyses: baseline C_{Cr}, change in C_{Cr} at one year after PT (except when this was the dependent variable), baseline Vv(Int/Cortex), CsA doses and levels at different times (average first month, first 3 months, first 6 months, first 12 months after PT and during the 5 years of the study). Four models with four different dependent variables were considered: (1) change in GFR at one year, (2) change in Vv(Int/Cortex) at five years, (3) change in % sclerosed glomeruli at five years, and (4) change in tubular athrophy score at five years.

Results

Clinical parameters

HbA1 became normal in all the PT patients (from 10.5 ± 1.4 at baseline to 6.6 ± 0.7% at five years, $P < 0.0001$), with no changes in the comparison group (from 11.3 ± 1.9 to 11.8 ± 2.2%). Creatinine clearance (C_{Cr}) decreased at five years in the transplanted patients ($P < 0.0001$, Fig. 2) and did not change significantly in the comparison group (102 ± 21 at baseline and 91 ± 26 ml/min/1.73 m^2 at five years, NS). The decline in C_{Cr} was significant in the first year in the PT patients but did not change thereafter (Fig. 2). The magnitude of the decline in C_{Cr} from baseline to one year in the PT patients correlated with the mean daily CsA dose during the twelfth post-PT month ($r = 0.79$, $P < 0.002$), as well as CsA blood levels during this month ($r = 0.75$, $P < 0.004$; Fig. 3). Similarly, the % decline from baseline in C_{Cr} in the first year correlated with both twelfth month mean CsA dose ($r = 0.62$, $P < 0.03$) and blood levels ($r = 0.75$, $P < 0.004$). By stepwise multiple regression analyses (model 1), CsA dose during the first year ($P < 0.04$) and CsA dose during the first six months ($P < 0.03$) were significant in determining the change in C_{Cr} at one year. Serum creatinine increased after PT from 0.95 ± 0.28 at baseline to 1.38 ± 0.61 mg/dl at five years ($P < 0.004$), but did not change in the comparison group (baseline 0.88 ± 0.14 and follow-up 0.88 ± 0.18 mg/dl). AER was not significantly different after PT [120 (7–2900) at baseline and 40 (2–2860) mg/24 hr at 5

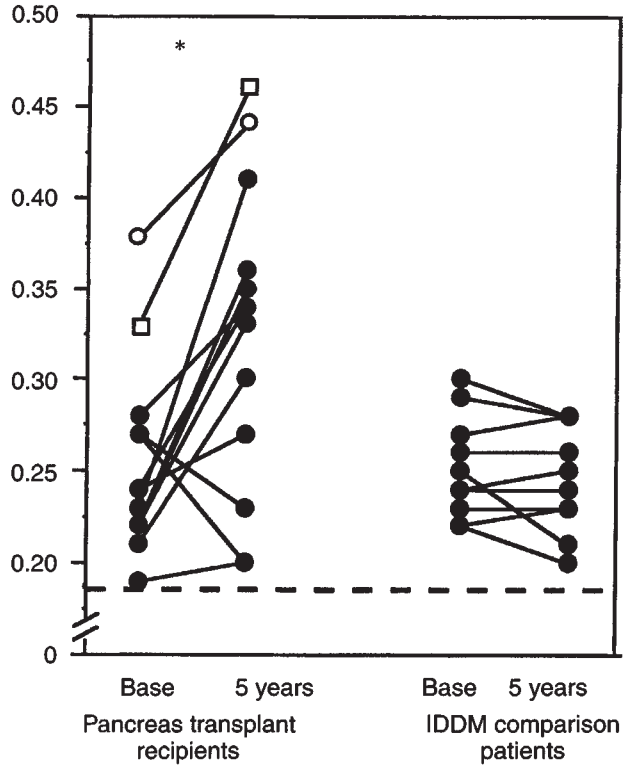

Fig. 4. *Interstitial fractional volume in the PT recipients and in the comparison patients at baseline (base) and follow-up (five years).* The dashed line represents the upper limit of normal in our laboratory (0.19). The asterisk indicates that interstitial fractional volume increased significantly following PT ($P < 0.005$); it was unchanged in the comparison patients.

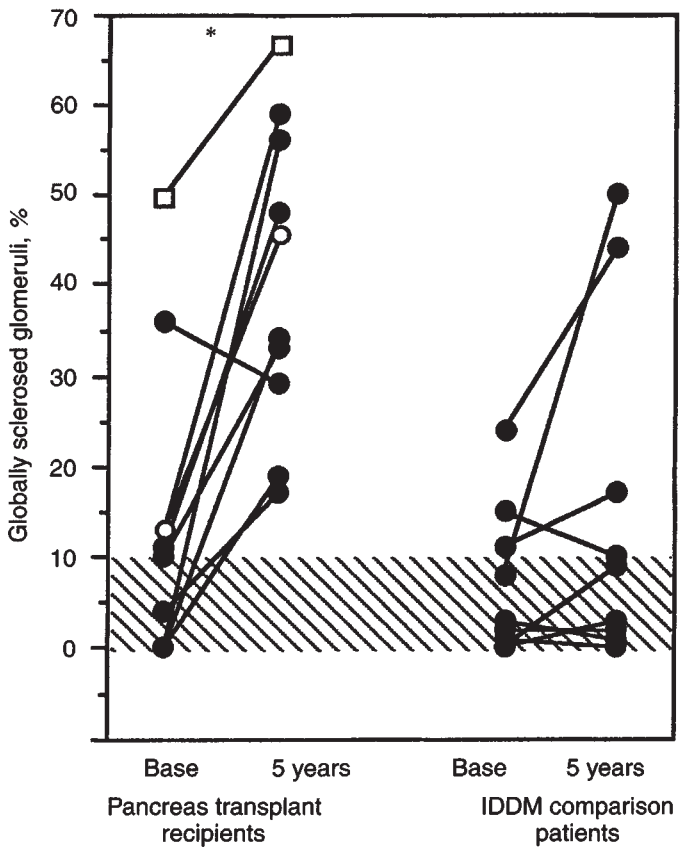

Fig. 5. *Sclerosed glomeruli (%) in the PT recipients and in the comparison patients at baseline (base) and follow-up (five years).* The dashed area represents the normal range in our laboratory (<10%). The asterisk indicates that the percent of globally sclerosed glomeruli increased significantly following PT ($P < 0.005$); it was unchanged in the comparison patients.

years, NS], and tended to increase in the comprison group [12 (2–280) at baseline and 19 (5–2462) mg/24 hr at 5 years, $P < 0.06$]. Mean BP also did not change in either group (91 ± 8 at baseline and 92 ± 10 mm Hg at 5 years in PT group; 85 ± 6 at baseline and 85 ± 7 mm Hg at 5 years in the comparison group). However, the number of patients treated for hypertension increased from 2 to 11 in the PT group, and from 1 to 4 in the comparison group.

Morphometric findings

The glomerular morphometric data have been previously reported [2]. Vv(Int/Cortex) increased after PT, but remained essentially unchanged in the comparison group (Fig. 4). No increase in Vv(Int/Cortex) was measured in the two year biopsies (0.27 ± 0.04 at two years vs. 0.26 ± 0.05 at baseline). Also there was no significant increase in interstitial fibrosis scores between the baseline (0.82 ± 0.91) and two year biopsies (1.11 ± 0.87). However, four PT patients had a more than 25% increase in Vv(Int/Cortex) and four a more than 0.5 grade increase in the interstitial fibrosis scores over this time. Increased Vv(Int/Cortex) was evident five years after PT (0.33 ± 0.08, $P < 0.004$ vs. baseline, $P < 0.04$ vs. 2 year follow-up). The interstitial fibrosis score increased significantly at five years (2.14 ± 1.18, $P < 0.003$ vs. baseline, $P < 0.004$ vs. 2 years). Vv(Int/Cortex) and the interstitial fibrosis score were highly significantly correlated ($r = 0.90$, $P < 0.0001$). There was no change in Vv(Int/Cortex) in five years in the comparison patients (0.25 ± 0.03 vs. 0.25 ± 0.03; Fig. 4).

Similarly, the index of tubular atrophy increased in five years in the PT group (24 ± 23 vs. 11.3 ± 13 at baseline, $P < 0.007$), but did not change significantly in the comparison group (11.1 ± 7.1 vs. 7.5 ± 6.6 at baseline, $P < 0.09$). The increase in tubular atrophy tended to correlate with the increase in Vv(Int/Cortex) over five years but this did not reach statistical significance ($r = 0.6$, $P < 0.07$).

Percent sclerosed glomeruli was 12.7 ± 17.1% at baseline, 16.2 ± 12.6% at two years (NS), and 39.8 ± 27.8% at five years post-PT ($P < 0.002$ vs. baseline and $P < 0.004$ vs. 2 years; Fig. 5). No significant change in percent sclerosed glomeruli was observed over five years in the controls (13.2 ± 18.8% vs. 6.2 ± 8.2% at baseline, NS).

Arteriolar hyalinosis scores were stable over the five years of the study in the PT patients (0.35 ± 0.25 vs. 0.37 ± 0.14 at baseline, NS), but increased in the comparison patients (0.56 ± 0.20 at five years vs. 0.36 ± 0.11 at baseline, $P < 0.03$).

CsA-associated arteriolopathy lesions were rare (<5% of all arterioles present in the bipsies) and were difficult to differentiate from diabetic arteriolar lesions. Although CsA-associated arteriolopathy lesions appeared to be mainly found in pancreas transplant patients receiving CsA, indistinguishable lesions were present in some baseline biopsies or in follow-up biopsies of comparison patients who had not received CsA. Thus these data were not statistically analyzed.

The increase from baseline in Vv(Int/Cortex) over five years in

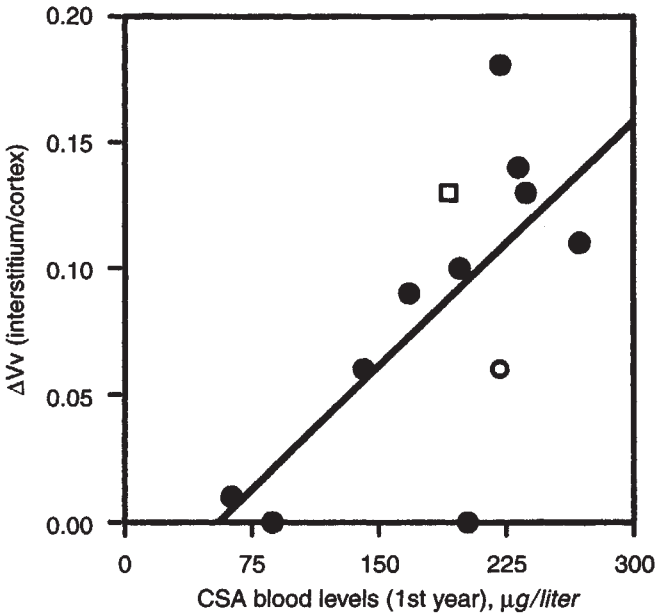

Fig. 6. *Correlation between CSA blood levels during the first year post-PT (μg/liter) and changes in Vv(interstitium/cortex) from baseline to five years after PT (r = 0.62, P < 0.03).*

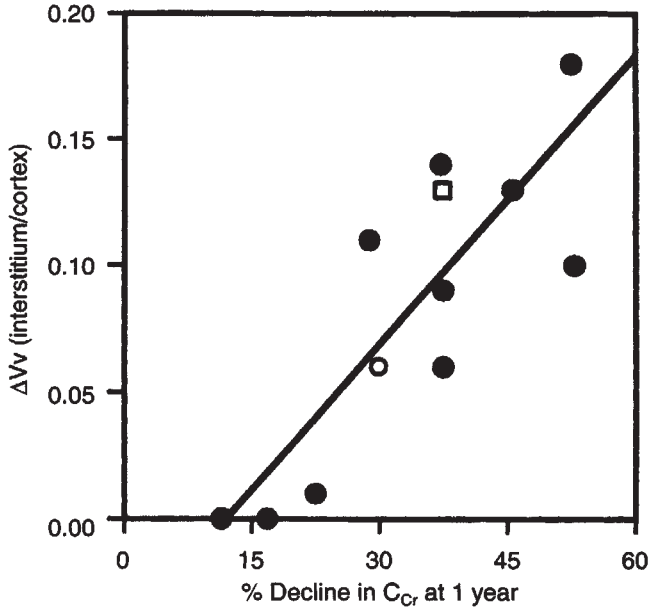

Fig. 7. *Correlation between the magnitude of decline in creatinine clearance (C_{Cr}) during the first year post-PT (expressed as % change over baseline values) and changes in Vv(interstitium/cortex) from baseline to five years after PT (r = 0.80, P < 0.003).*

the PT patients correlated with both the mean daily CsA dose in the first month ($r = 0.6$, $P < 0.05$), and with the mean daily CsA dose in the first post-transplant year ($r = 0.62$, $P < 0.04$). As well, the mean of CsA blood levels in the first year correlated directly with the increase from baseline in Vv(Int/Cortex) over five years in the PT patients ($r = 0.68$, $P < 0.03$; Fig. 6). Similar correlations were observed when the increase in interstitium in five years was expressed as % over baseline.

The strongest predictor of the increase in Vv(Int/Cortex) over five years was the decline in GFR in the first year ($r = 0.76$, $P < 0.005$); similarly the % decline in GFR in the first year correlated with the % increase in Vv(Int/Cortex) ($r = 0.8$, $P < 0.003$; Fig. 7).

By stepwise multiple regression analysis, change in C_{Cr} at one year ($P < 0.03$) and CsA blood levels in the first three months ($P < 0.03$) were significant in determining the change in Vv(Int/Cortex) at five years. None of the variables was significant when change in % sclerosed glomeruli at five years or change in tubular atrophy at five years were considered as the dependent parameters.

Discussion

This study demonstrates that renal lesions known to be associated with long-term CsA administration develop in the native kidneys of IDDM PT recipients. The severity of these lesions in the PT recipients after five years of CsA therapy was strongly predicted by CsA blood levels, CsA dose, and magnitude of the decline in C_{Cr} during the first post-transplant year. Additionally, this study showed that the adverse effects of CsA on renal structure could be missed if examined after only two years of CsA therapy. The study design was important in allowing us to make these observations: in this study C_{Cr} and renal biopsies were performed before the administration of CSA and two and five years later, as determined by protocol, not by clinical events. This is also the first study in non-kidney transplant patients [21] where all the patients on CSA therapy were studied for the prolonged duration of five years. An additional advantage of the study's design was the availability of GFR and kidney biopsies, performed five years apart, in a comparison group, with the same underlying systemic disease (IDDM).

Recently we reported, in the same patients as presented here, that five years of euglycemia resulting from successful pancreas transplantation were unable to reverse established diabetic glomerular structural changes in long-term IDDM patients [2].

The finding of increased interstitial fibrosis, tubular atrophy, and global glomerular sclerosis in CsA-treated PT recipients, as opposed to the stability of these lesions over five years in the comparison patients, indicates that these lesions are consequences of CsA administration, and not of the preexisting diabetic state in PT recipients. This view is strongly supported by the observations that the severity of interstitial expansion over five years and CsA blood levels and dosages were highly significantly correlated.

Renal morphological changes induced by CsA have been extensively described [4–10]. CsA nephrotoxicity is said to be characterized by tubulointerstitial lesions of focal (striped) interstitial fibrosis associated with tubular atrophy within the areas of fibrosis. Arteriolar alterations including ring or nodular hyalinosis, intimal fibrosis and mucoid thickening of the intima have also been described [7].

Glomerular changes have not often been reported. However, focal segmental glomerulosclerosis has been described in renal allografts in conjunction with CsA-associated arteriolopathy [22]. Also, increased numbers of obsolescent glomeruli have been seen in cases of advanced interstitial fibrosis and tubular atrophy in the native kidneys of CsA patients [5, 7, 10].

In this study we concentrated on the interstitial, tubular, and glomerulosclerotic lesions since diabetic- and CsA-associated arteriolopathy may, as confirmed here, be impossible to differentiate structurally [7]. Also, CsA-associated arteriolar lesions can

regress upon CsA dose reduction [23], and our first biopsies after PT in CsA-treated patients were performed only after two years, one year after the major CsA dose reductions were made. Arteriolar hyalinosis lesions increased in IDDM control patients, but remained stable after PT. It is possible that PT prevented progression of these diabetic lesions, or even ameliorated them, and that arteriolar hyalinosis changes due to CsA were not pronounced enough to outweigh this beneficial effect.

The average increase in Vv(Int/Cortex) of 23% and the increase in the frequency of sclerotic glomeruli from 13 ± 17% to 40 ± 28% in the PT represents a serious deterioration in renal structure which, if progressive, could foretell irreversible renal functional deterioration. As mentioned, we found direct correlations between the five year increase in the interstitial space, measured as Vv(Int/Cortex), and measures of CsA blood levels and doses during the first post-PT year but not thereafter. The majority of the PT patients were treated with CsA doses which have been considered to be "high" (>5 mg/kg/day) [24]. Therefore, we could not separately analyze patients on "low" and "high" CsA dose (< and >5 mg/kg/day) as has been done in previous studies [24]. Nonetheless, our linear regression analyses did not indicate a threshold effect for CsA-induced renal injury at mean doses between 3 and 12 mg/kg/day during the first year. Rather, there was a progressive worsening in measures of interstitial expansion with increasing CsA dose.

The best predictor of the five-year increase in Vv(Int/Cortex) was the degree of decline in C_{Cr} after one year of CsA therapy. It is possible that the decline in C_{Cr} at shorter times after the institution of CsA therapy could be a better predictor; however, our patients were regularly evaluated by protocol only after one year following PT. The decline in C_{Cr} at one year post-PT was closely related to the CsA dose and blood levels at the same time (mean of 12th month dose and level values). Thus, it is not surprising that average CsA blood levels and dose during the first year were also strongly predictive of the increase in interstitial fibrosis over five years. Tomlanovich et al have reported that C_{Cr} systematically underestimates the decline in glomerular filtration rate (GFR) as measured by inulin clearance, particularly if the inulin GFR is less than 60 ml/min/1.73 m^2 [25]. This observation would serve to strengthen the findings of the current study which used C_{Cr} to estimate GFR.

Several studies have investigated the relationships between CSA dose and/or blood levels and changes in renal function and structure [23, 26–28]. These studies, however, lacked baseline biopsies for evaluating changes in lesions over time and often lacked a control group with the same underlying disease. Thus, when kidney biopsies were analyzed in patients with psoriasis either untreated with CsA or treated with low dose CsA for a mean of 15 months, similar minimal or slight interstitial and vascular changes were observed in both groups [13]. These results emphasize the importance of baseline or control biopsies. Also, in these studies the range of duration of CsA therapy was very wide and the follow-up of the patients was much shorter than in our study. A correlation between CsA dose (but not blood levels) and CsA-induced renal lesions has been described by Feutren and Mihatsch [24] and Svenson, Bohman and Hallgren [26], but not by others [14, 23, 27, 28]. In the present study CsA blood levels in the first year were even stronger predictors than CsA dose for changes in interstitium five years after PT. This makes sense considering patient variability in absorption and turnover of CsA as well as possible variability in compliance. Also, associations of blood levels and outcomes such as changes in renal function and structure may be easier to observe when blood levels are obtained at fixed times after CsA dosing and are measured in a single laboratory. As well, these relationships of CsA dose and level to decline in GFR and to severity of lesions, may be more apparent when there is more variation in CsA dose among the patients. This may explain why, despite baseline observations and appropriate controls, these relationships were not detected in CsA-treated psoriasis patients [14].

We did not find progression of interstitial expansion in the two year biopsies as compared to baseline biopsies in PT patients; these findings contrast with the majority of earlier reports describing CsA-induced lesions after short-term treatment [9, 10, 14, 24, 29, 30]. For example, Zachariae et al [30] compared kidney biopsies performed before and after one year of low-dose CsA therapy in psoriatic patients, and found a slight but significant increase in interstitial expansion. Similarly, in a well-designed study with appropriate controls, Young et al described tubulointerstitial lesions in psoriasis patients after one year of CsA therapy, and these lesions progressed over the subsequent two years of CsA treatment [14]. We do not have a clear understanding of these discrepant results. One possibility is that underlying diabetic interstitial lesions of variable severity may have made subtle CsA changes at two years more difficult to detect than in the above studies, where kidney structure was closer to normal at baseline [14, 30]. Another possibility is that normoglycemia following PT induced a relative decrease in the volume of the interstitial compartment, offsetting any early increase caused by CsA. A third possibility is that of methodologic differences in the measurement of the lesions. However, whether we did not find changes at two years because they were not present or because they were not measurable, one of the main conclusions of the present study is that there was progression of CsA related lesions between the second and the fifth year of treatment, despite reduced CsA dose and levels during these later years. Along these lines, Habib and Niaudet [31] also reported that some children on long-term CsA therapy for idiopathic nephrosis had little or no CsA-related renal lesions on initial (average 13 months) post-treatment biopsy, but showed important lesions in subsequent (average 29 months) biopsies.

The value of early changes in serum creatinine or C_{Cr} in predicting early CsA renal structural changes has been previously described [24, 26, 30]. However, this is the first study addressing whether early renal functional changes predict late CsA structural changes. The present study demonstrates that a change in C_{Cr} at one year is a strong predictor of the development of late interstitial lesions. Thereafter tubulointerstitial lesions progress, despite stable C_{Cr} [32]. Thus stable C_{Cr} in CsA-treated patients cannot be interpreted as indicating a lack of progressive renal injury.

Our studies also describe progression of the lesion of global glomerulosclerosis over five years in the PT recipients. Similar findings have been described in CsA-treated heart transplant [5] and uveitis [10] patients. These are important renal structural changes which may independently contribute to ultimate deterioration in renal function. The increase in global glomerulosclerosis may not have been noted in many short-term studies because it appears to require a longer time to become evident.

In summary, these studies document the development of important interstitial, tubular, and glomerular lesions in the native kidneys of PT patients treated with CsA. The interstitial lesions were closely related to early CsA blood levels and effects of CsA

on depression of GFR. PT does not result in amelioration of specific lesions of diabetic glomerulopathy in five years [2], but is associated with marked worsening of overall renal structure based upon these CsA-induced changes. Thus, so long as nephrotoxic drugs remain a component of the immunosuppressive protocols, PT may negatively impact on the kidney. PT in patients with native kidneys should, therefore, only be considered for debilitating or lifethreatening metabolic instability [33]. Similar "cost-benefit" analyses applied to other relative indications for long-term CsA therapy such as psoriasis, uveitis, and rheumatoid arthritis, would suggest that only the most severe cases should be selected for long-term CsA treatment.

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References

1. SUTHERLAND DER, KENDALL DM, MOUDRY KC, NAVARRO X, KENNEDY WR, RAMSAY RC, STEFFES MW, MAUER SM, GOETZ FC, DUNN DL, NAJARIAN JS: Pancreas transplantation in nonuremic, type I diabetic recipients. *Surgery* 104:453–464, 1988
2. FIORETTO P, MAUER SM, BILOUS RW, GOETZ FC, SUTHERLAND DER, STEFFES MW: Effects of pancreas transplantation on glomerular structure in insulin-dependent diabetic patients with their own kidneys. *Lancet* 342:1193–1196, 1993
3. FIORETTO P, MOGENSEN CE, MAUER SM: Diabetic nephropathy, in *Pediatric Nephrology*, edited by HOLLIDAY MA, BARRATT TM, AVNER ED, New York, Williams and Wilkins, 1994, pp 576–585
4. MIHATSCH MJ, THIEL G, RYFFEL B: Hystopathology of cyclosporine nephrotoxicity. *Transplant Proc* 20(Suppl 3):759–771, 1988
5. MYERS B, ROSS J, NEWTON L, LEUTSCHER J, PERLROTH M: Cyclosporine-associated chronic nephropathy. *N Engl J Med* 311:699–705, 1984
6. BERTANI T, FERRAZZI P, SCHIEPPATI A, RUGGENENTI P, GAMBA A, PARENZAN L, MECCA G, PERICO N, IMBERTI O, REMUZZI A, REMUZZI G: Nature and extent of glomerular injury induced by cyclosporine in heart transplant patients. *Kidney Int* 40:243–250, 1991
7. MIHATSCH MJ, THIEL G, RYFFEL B: Renal side-effects of cyclosporine A with special reference to autoimmune diseases. *Brit J Dermatol* 122(Suppl 36):101–115, 1990
8. SIBLEY RK, RYNASIEWICZ J, FERGUSON RM, FRYD D, SUTHERLAND DER, SIMMONS RL, NAJARIAN JS: Morphology of cyclosporine nephrotoxicity and acute rejection in patients immunosuppressed with cyclosporine and prednisone. *Surgery* 94:225–234, 1983
9. MIHATSCH MJ, THIEL G, BASLER V, RYFFEL B, LAMDMANN J, VON OVERBECK J, ZOLLINGER HU: Morphological patterns in cyclosporine-treated renal transplant recipients. *Transplant Proc* 17(Suppl 1):101–116, 1985
10. PALESTINE AG, AUSTIN HA, BALOW JE, ANTONOVYCH TT, SABNIS SG, PREUSS HG, NUSSENBLATT RB: Renal histopathologic alterations in patients treated with cyclosporine for uveitis. *N Engl J Med* 314:1293–1298, 1986
11. GAFTER U, KALECHMAN Y, ZEVIN D, KORZETS A, LIVNI E, KLEIN T, SREDNI B, LEVI J: Tubulointerstitial nephritis and uveitis: Association with suppressed cellular immunity. *Nephrol Dial Transplant* 8:821–826, 1993
12. MADEDDU P, ENA P, GLORIOSO N, CERIMELE D, RAPPELLI A: High prevalence of microproteinuria, an early index of renal impairment, in patients with diffuse psoriasis. *Nephron* 48:222–225, 1988
13. International kidney biopsy registry of cyclosporine A in autoimmune diseases. Kidney biopsies in control or cyclosporin A treated psoriatic patients. *Brit J Dermatol* 122(Suppl 36):95–100, 1990
14. YOUNG W, ELLIS CN, MESSANA JM, JOHNSON KJ, LEICHTMAN AB, MIHATSCH MJ, HAMILTON TA, GROISSER DS, FRADIN MS, VOORHESS JJ: A prospective study of renal structure and function in psoriasis patients treated with cyclosporin. *Kidney Int* 46:1216–1222, 1994
15. BOWERS LD, CANAFAX DM, SINGH J, SIEFELDIN R, SIMMONS RL, NAJARIAN JS: Studies of cyclosporine blood concentrations: Analysis, clinical utility, pharmacokinetics, metabolites and chronopharmacology. *Transplant Proc* 18(Suppl 5):137–143, 1986
16. ELLIS EN, BASGEN JM, MAUER SM, STEFFES M. W: Kidney biopsy technique and evaluation, in *Methods in Diabetes Research, Volume II: Clinical Methods*, edited by CLARKE WL, LARNER J, POHL SL, New York, John Wiley & Sons, 1986, pp 633–647
17. LANE PH, STEFFES MW, FIORETTO P, MAUER SM: Renal interstitial expansion in insulin-dependent diabetes mellitus. *Kidney Int* 43:661–667, 1993
18. MAUER SM, STEFFES MW, ELLIS EN, SUTHERLAND DER, BROWN DM, GOETZ FC: Structural-functional relationships in diabetic nephropathy. *J Clin Invest* 74:1143–1155, 1984
19. HARRIS RH, STEFFES MW, SUTHERLAND DER, MAUER SM: Global glomerular sclerosis and arteriolar hyalinosis in insulin dependent diabetes. *Kidney Int* 40:107–114, 1991
20. MIHATSCH MJ, ANTONOVYCH T, BOHMAN S-O, HABIB R, HELMCHEN U, NOEL LH, OLSEN S, SIBLEY RK, KEMENY E, FEUTREN G, MIHATSCH MJ: Cyclosporin A nephropathy: Standardization of the evaluation of kidney biopsies. *Clin Nephrol* 41:23–32, 1994
21. WILCZEK H, BOHMAN S-O, KLINTMALM G, GROTH CG: Five-year serial renal graft biopsy study in cyclosporine-treated patients. *Transplant Proc* 30(Suppl 3):812–815, 1988
22. TAKEDA A, MOROZUMI K, UCHIDA K, YOKOYAMA I, TAKAGI H, YOSHIDA A, FUJINAMI T, THIEL G, GUDAT F: Is Cyclosporine-associated glomerulopathy a new glomerular lesion in renal allografts using CyA? *Transplant Proc* 25:515–517, 1993
23. MOROZUMI K, THIEL G, ALBERT FW, BANFI G, GUDAT F, MIHATSCH MJ: Studies on morphological outcome of cyclosporine-associated arteriolopathy after discontinuation of cyclosporine in renal allografts. *Clin Nephrol* 38:1–8, 1992
24. FEUTREN G, MIHATSCH J: Risk factors for cyclosporine induced nephropathy in patients with autoimmune dieseases. *N Engl J Med* 326:1654–1660, 1992
25. TOMLANOVICH S, GOBLEZ H, PERLROTH M, STINSON E, MYERS BD: Limitations of creatinine in quantifying the severity of cyclosporine-induced chronic nephropathy. *Am J Kidney Dis* 8:332–337, 1986
26. SVENSON K, BOHMAN S-O, HALLGREN R: Renal interstitial fibrosis and vascular changes. Occurrence in patients with autoimmune diseases treated with cyclosporine. *Arch Intern Med* 146:2007–2010, 1986
27. DIETERLE A, GRATWOHL A, NIZZE H, HUSER B, MIHATSCH MJ, THIEL G, TICHELLI A, SIGNER E, NISSEN C, SPECK B: Chronic cyclosporine-associated nephrotoxicity in bone marrow transplant patients. *Transplant* 49:1093–1100, 1990
28. BACH JF, FEUTREN G, HANNEDOUCHE T, LANDAIS P, TIMSIT J, BOITARD CH, BOUGNERES P, BOITARD C, GRUNFELD JP, ASSAN R: Factors predictive of cyclosporine-induced nephrotoxicity: The role of cyclosporine blood levels. *Transplant Proc* 22:1296–1298, 1990
29. SUND S, FØRRE O, BERG KJ, KVIEN TK, HOVIG T: Morphological and functional renal effects of long-term low-dose cyclosporin A treatment in patients with rheumathoid arthritis. *Clin Nephrol* 41:33–40, 1994
30. ZACHARIAE H, HANSEN HE, KRAGBALLE K, OLSEN S: Morphologic renal changes during cyclosporine treatment of psoriasis. *J Am Acad Dermatol* 26:415–419, 1992
31. HABIB R, NIAUDET P: Comparison between pre- and post-treatment renal biopsies in children receiving cyclosporine for idiopathic nephroses. *Clin Nephrol* 42:141–146, 1994
32. DE FRANCISCO AM, MAUER SM, STEFFES MW, GOETZ FC, NAJARIAN JS, SUTHERLAND DER: The effect of cyclosporine on renal function in non-uremic diabetic recipients of pancreas transplants. *J Diab Compl* 1:128–131, 1987
33. REMUZZI G, RUGGENENTI P, MAUER SM: Pancreas and kidney/pancreas transplantation: Experimental medicine or real improvement? *Lancet* 343:27–31, 1994