Cyclosporine-Induced Chronic Nephropathy: An Obliterative Microvascular Renal Injury

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ABSTRACT
Physiologic and morphologic techniques have been used to study kidneys of 200 cardiac transplant recipients treated with either low- or high-dose cyclosporine. After 12 months, both low- (4.6 ± 0.4) and high-dose cyclosporine (6.3 ± 0.3 mg/kg/24 h; P < 0.01) were associated with depression of glomerular filtration rate below values in a third group of 100 recipients never exposed to cyclosporine by 40 to 47%. Determination of renovascular pressures and flows as well as analysis of transglomerular sieving of dextrans revealed renal vascular resistance in cyclosporine-treated recipients to be elevated >twofold, due largely to an increase in preglomerular resistance. Morphologic changes in renal tissue of both cyclosporine groups included an occlusive afferent arteriolopathy with downstream collapse or sclerosis of glomeruli. Ischemic nephrons were associated with patchy fibrosis of the surrounding interstitium. Follow-up for up to 9 yr reveals persistent but stable azotemia, on average. Longitudinal physiologic studies over a 48-month period (N = 15) during which cyclosporine was reduced in dosage (to 3.1 ± 0.3 mg/kg) or withdrawn revealed a persistently reduced but constant level of glomerular filtration rate. Increasing ischemic glomerular collapse and sclerosis were observed at repeat renal biopsy. Remnant (spared) glomeruli exhibited hypertrophy; presumably elevated single nephron glomerular filtration rate maintained two-kidney glomerular filtration rate constant despite the declining fraction of functional glomeruli. By actuarial analysis, the cumulative incidence of end-stage renal failure in cardiac transplant recipients treated at this institution from 1980 onwards with continuous cyclosporine therapy has reached 10%. It was concluded that continuous treatment with cyclosporine for more than 12 months causes progressive injury to renal microvessels that is rarely reversible and has the potential to advance to end-stage renal failure.

Key Words: Afferent arteriolopathy, glomerular morphometry, glomerular dynamics, segmental renovascular resistances, progression to end-stage renal failure

A decade has elapsed since cyclosporine (CsA) was introduced into the immunosuppressive regimen of patients receiving cardiac transplants (Tx) at our institution. Five-year graft survival has increased to 68% compared with only 45% in the preceding decade. This salutory effect has been marred to some extent by the finding that many cardiac Tx recipients have developed a chronic renal injury when on continuous CsA therapy. The purpose of this report is to review a series of investigations into the pathophysiology of this injury, which we have conducted over the past 8 yr (1–4). In the absence of a convincing analog in experimental animals, our observations provide some useful insights into the pathogenesis of CsA-induced chronic nephropathy (CICN) and into the manner in which it progresses.

METHODS

Study Populations
Observations were made in two successive CsA-treated cohorts of cardiac Tx recipients, each containing 100 subjects. The first cohort, transplanted between December 1980 and April 1984, received CsA in relatively high dosage compared with the second cohort, which was transplanted between April 1984 and January 1986 (Figure 1). The therapeutic goal was to maintain trough serum levels of immunoassayable CsA between 100 and 300 ng/mL in the first cohort and between 50 and 150 ng/mL in the second cohort. A third cohort of 100 consecutive cardiac Tx recipients transplanted between April 1976 and December 1980, and never exposed to CsA, served as a control group. Age and the underlying etiology of the native cardiac diseases were similar in the three cohorts.
FIGURE 1. Mean (±SE) of CsA dosage versus time in high- (open circles) and low-dose (closed circles) CsA cohorts. P refers to concomitant prednisone and A to azathioprine therapy.

Physiologic Determinations

Subsets of each of the foregoing cohorts (N = 21, 14 and 23, respectively) were selected for detailed physiologic studies on the basis of consecutive admissions to a clinical research center for routine yearly evaluation of allograft performance. CsA-treated subjects were 12 months and non-CsA-treated controls were 48 to 60 months post-Tx. They were studied after giving consent to a protocol that had been approved previously by the Institutional Review Board at Stanford University.

A clearance technique was used to determine the rates of renal blood flow (RBF) and glomerular filtration (GFR) in each subject. RBF was estimated from the clearance of p-aminohippuric acid (PAH), the fractional hematocrit, and the renal extraction ratio of PAH. GFR was equated with the clearance of micropuncture determinations in the rat which show that proximal tubule pressure exceeds first order peritubular capillary pressure (estimated here as RVPw) by 3 to 5 mm Hg and is uninfluenced by CsA therapy (9,10).

Morphologic Determinations

Ten consecutive subjects, seven from the high-dose and three from the low-dose CsA groups, were subjected to a renal biopsy at the time of their first routine annual evaluation, 12 months post-Tx. These biopsies were performed the day after clearance studies, but the corresponding level of renal injury was unknown to the pathologist at the time of histopathologic examination. In addition to routine light and electron microscopy, all glomeruli in each biopsy core were subjected to computerized area perimeter analysis at a magnification of X1,000, as described elsewhere (2,3). The ultrastructure of two glomeruli from each core was also examined by area perimeter analysis at a magnification of X4,000 (2). Renal biopsy tissue from 10 living transplant donors provided control values for the morphometric quantities of interest.

Longitudinal Examination

Fifteen members of the high-dose CsA cohort, examined initially after 12 or 24 months of CsA therapy, were restudied with identical physiologic techniques at the time of routine 36- and 48-month evaluation. Six of these were also subjected to a second renal biopsy on the basis of constancy of prevailing serum creatinine levels (apparent non-progressors).
Sporadic or serial clearance determinations were also performed in 10 individuals who exhibited progressive azotemia and went on to develop end-stage renal failure (ESRF) during the course of our decade-long observations. A renal biopsy was performed during the period of declining renal function in six instances. The course of progressive renal injury was also monitored in five recipients of heart-lung Tx who developed ESRF in association with continuous CsA therapy.

RESULTS

Clinical Features of Early CICN

 Prevailing serum creatinine levels in the three cohorts are plotted as a function of time for the first 3 yr post-Tx in Figure 2. The level of serum creatinine in the early pre-1980 cohort never exposed to CsA (no CsA) remained in the normal range. In contrast, serum creatinine levels increased progressively during the first 6 post-Tx months in each CsA-treated cohort and have remained chronically elevated ever since. The elevation is more marked with high-CsA dosage (high-CsA) than with low-CsA dosage (low-CsA), to approximately 2.1 versus 1.7 mg/dL. Nevertheless, it is apparent that cardiac Tx recipients suffer a chronic renal injury when treated continuously with CsA, either in high or low dosage.

In addition to azotemia, a second major clinical finding associated with CICN was arterial hypertension. Whereas only 3% of patients in the no-CsA cohort required antihypertensive therapy within the first post-Tx year, greater than 80% of the high- and low-CsA cohorts required such therapy during the corresponding period. Antihypertensive treatment notwithstanding, mean arterial pressure during the 12 month follow-up visit was elevated on average by 17 and 16 mm Hg in the high- and low-CsA cohorts versus the no-CsA cohort. The CICN was also accompanied by low-grade proteinuria. Although dipstick testing was usually negative, sensitive immunochromatographic techniques revealed the rates of urinary albumin and immunoglobulin excretion to be elevated into a pathologic, albeit subclinical range (so-called microalbuminuria).

Pathophysiology of Early CICN

Hemodynamic Findings. The renovascular pressures, flows, and resistances prevailing after 12 months of CsA are summarized in Table 1 and Figure 3. Whereas the GFR in no-CsA controls was within the normal range (95 ± 4), treatment with either high- or low-dose CsA lowered the GFR by almost half (50 ± 5 and 56 ± 3 mL/min/1.73 m², respectively). Determination of the renal PAH extraction ratio during right-sided cardiac catheterization (0.89 ± 0.06 in the no-CsA group versus 0.78 ± 0.13 in the CsA-
treated groups; \( P < 0.01 \) permitted RBF to be determined with precision. It was lowered below the control value by \( \sim 40\% \) in each CsA group. By contrast, cardiac output did not differ between the two groups. It follows that the fraction of cardiac output perfusing the kidneys was markedly depressed after 12 months of CsA, averaging only 9 \( \pm 1\% \) in the two CsA-treated groups versus 16 \( \pm 1\% \) in no-CsA controls. This points to a disproportionate elevation of resistance in the renal versus the systemic vascular bed. Calculated total RVR was similar in the high- and low-CsA groups, exceeding that in no-CsA controls by more than twofold (Table 1).

The prevailing hydraulic pressures along the renovascular bed were determined during the course of routine catheterization of the left and right chambers of the allografted heart. The findings in the low-CsA group are compared with those of no-CsA controls in Figure 3. \( \overline{AP} \) was elevated to 116 \( \pm 3 \) in the former versus only 100 \( \pm 2 \) mm Hg in no-CsA controls. As computed from their dextran sieving profiles, exactly the opposite was true of glomerular capillary pressure, which is estimated to have been lower in the CsA-treated group—54 versus 59 mm Hg, respectively (3). In contrast, the pressure drop beyond the glomeruli in the two groups was almost identical, falling from about 15 mm Hg in peritubular capillaries (renal vein occlusion pressure) to 5 or 6 mm Hg in the right atrium (Figure 3).

Knowledge of these axial pressures along with the renovascular flows in Table 1 permits an analysis of segmental resistance by using equations 2 and 3. In the no-CsA controls, preglomerular RVR is estimated to be 53 (mm Hg·min)/L, that is, 39% of total RVR. In keeping with the steeper pressure drop from aorta to glomerular capillaries, preglomerular RVR was elevated by threefold to 158 (mm Hg·min)/L in the low-CsA group, comprising 56% of total RVR. Postglomerular RVR was also higher in the low-CsA group than in controls, but the disparity was much smaller—122 versus 88 (mm Hg·min)/L. It follows that the >twofold elevation of total RVR observed with chronic CsA therapy is caused predominantly by a disproportionate rise in preglomerular resistance.

**Morphological Findings.** The major resistance vessel in the preglomerular circulation is the afferent arteriole, and, in keeping with the marked elevation in its resistance, this vessel also exhibited conspicuous histopathologic alterations. By light microscopy, endothelial cells were seen to be swollen and exhibited mucoid degeneration, whereas myocytes could frequently be seen to have undergone necrosis (2). The most pronounced change was hyalinosis of the arteriolar wall. By electron microscopy, the hyalin material appeared electron dense and could be seen to spare the subendothelial layer of the wall, while infiltrating the medial layer, often replacing the smooth muscle cells (for illustrative examples, see references 2 and 3). In advanced lesions, the hyalin material expanded the arteriolar wall leading to narrowing of the adjacent lumen. Of interest, the hyalinosis differed from that seen in hypertensive and diabetic kidney disease in that efferent arterioles were spared completely.

The remaining histopathologic abnormalities were consistent with patchy ischemic damage downstream from an occluded afferent arteriole. Approximately 15% glomeruli in the biopsy cross-sections were small and collapsed. The tubular cells of nephrons damaged by ischemia were atrophic, and their basement membranes thickened; the surrounding interstitium exhibited fibrosis. It should be emphasized that the constellation of glomerular, tubular, and interstitial changes were focal in nature. They were arrayed in "stripelike" zones and interspersed with broader zones of normal-appearing renal cortex. This stripelike distribution is consistent with localized ischemic damage to nephrons, which is consequent upon an upstream occlusive afferent arteriopathy (11).

The bell-shaped distribution of glomerular cross-sectional area (\( A_G \)) observed in healthy controls was lost in the CsA-treated recipients biopsied 12 months post-Tx, in whom the distribution of \( A_G \) was shifted to both smaller and larger sizes (Figure 4). Many of the glomeruli of \( A_G \leq 6,000 \mu^2 \) were those that had undergone ischemic collapse, whereas other unaffected glomeruli achieved \( A_G \geq 24,000 \mu^2 \), a phenomenon rarely observed in controls. The major ultrastructural alteration in noncollapsed glomeruli was...
an excess of mesangial extracellular matrix, such that the fractional mesangial area averaged 0.23 ± 0.02 versus only 0.17 ± 0.007 in controls ($P < 0.01$). Thus, notwithstanding the renal underperfusion that attended 12 months of CsA therapy, a subset of glomeruli appeared to have undergone compensatory hypertrophy and to have accumulated extracellular matrix in the mesangial region.

**Course of Late CICN**

Fifteen members of the high-CsA group were entered into a longitudinal study in 1983, at the time of routine yearly evaluation either 12 or 24 months post-Tx. Serial physiologic studies were performed at 36 and 48 months post-Tx (Table 2). Between the initial and terminal study, CsA was withdrawn in four subjects and the daily CsA dose was lowered from 6.8 to 3.1 mg/kg in the remainder. Nevertheless, GFR failed to improve, remaining essentially constant. Total RVR also failed to change significantly over the period of observation. The single physiologic determination that pointed to possible worsening of renal injury was a progressive increase in the excretion rates of albumin and IgG (Table 2).

Unequivocal evidence of progression of CICN was provided by a second renal biopsy performed 24 months after the initial biopsy in six subjects in whom serum creatinine levels and GFR had remained constant (Table 3). The percentage of glomeruli that had collapsed in the interim increased from 15 to 24%. An additional 15% of glomeruli in the repeat biopsy exhibited segmental or global sclerosis versus only 6% in the first biopsy. The increase in the percentage of obliterated glomeruli (collapse plus sclerosis) from 21 to 39% between the two biopsies was associated with more conspicuous afferent arteriopathy and greater alteration of the tubulointerstitial compartment (Table 3).

Once the fraction of glomeruli undergoing progressive obliteration has crossed a certain threshold, one would predict that the nephropathy is likely to become decompensated, leading to increasing azotemia and, eventually, ESRF. With the passage of a decade since CsA therapy was first instituted, it has now become apparent that this is indeed the case. Actuarial analysis reveals that only 90% of cardiac Tx recipients remain free of ESRF after 8 yr of therapy (Figure 5). A shorter follow-up in a smaller number ($N = 73$) of recipients of heart-lung Tx reveals an even higher cumulative incidence of ESRF. Only 89% are free of ESRF after 5 yr of therapy (4).

Analysis of the course of CICN in the 15 subjects who have progressed to ESRF to date reveals the renal injury to have the following characteristics. The time to onset of ESRF varies from 1 to 8 yr, and two stages of injury can usually be clearly identified. The first is a compensated stage during which GFR and serum creatinine levels remain constant. The findings in Table 3 suggest that such compensation occurs in the face of progressive obliteration of an increasing fraction of glomeruli. As illustrated by the two examples shown in Figure 6, withdrawal of CsA once the compensated stage of chronic injury is established results in either no improvement in (upper panel), or in only partial and transient reversal of, the level of azotemia (lower panel). Eventually the compensated stage gives way to a second stage of decompensation. Azotemia becomes progressive and leads eventually to ESRF, notwithstanding the complete withdrawal of CsA therapy (Figure 6).

Insights into the biphasic course of CICN is provided by the morphometric analysis of glomeruli obtained by biopsy during the decompensated stage in the first six patients to advance to ESRF. As shown

**TABLE 2. Serial physiologic determinations**

<table>
<thead>
<tr>
<th></th>
<th>12 or 24 (months)</th>
<th>36 (months)</th>
<th>48 (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GFR (mL/min/1.73 m²)</td>
<td>56 ± 4</td>
<td>54 ± 7</td>
<td>55 ± 5</td>
</tr>
<tr>
<td>RVR ((mm Hg·min)/L)</td>
<td>212 ± 8</td>
<td>265 ± 31</td>
<td>249 ± 40</td>
</tr>
<tr>
<td>UValbumin (µg/min)</td>
<td>162 ± 70</td>
<td>396 ± 164</td>
<td>546 ± 300</td>
</tr>
<tr>
<td>UVIgG (µg/min)</td>
<td>6 ± 2</td>
<td>21 ± 7</td>
<td>53 ± 43</td>
</tr>
</tbody>
</table>

* $p < 0.05$ versus initial study.

**TABLE 3. Serial biopsy findings**

<table>
<thead>
<tr>
<th>Glomerular ischemic collapse (%)</th>
<th>1st biopsy (12 or 24 months)</th>
<th>2nd biopsy (36 or 48 months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>% Glomerular ischemic collapse</td>
<td>14.7 ± 12.5</td>
<td>24.2 ± 13.5</td>
</tr>
<tr>
<td>% Sclerosed glomeruli</td>
<td>6.2 ± 6.7</td>
<td>15.3 ± 4.8</td>
</tr>
<tr>
<td>Afferent arteriopathy index</td>
<td>0.5 ± 0.5</td>
<td>1.8 ± 1.2</td>
</tr>
<tr>
<td>Tubulointerstitial index</td>
<td>1.5 ± 0.5</td>
<td>2.0 ± 0.6</td>
</tr>
</tbody>
</table>

* $p < 0.05$ versus initial biopsy.

* * *
in the lower panel of Figure 3, glomerular tufts have become shifted to smaller $A_0$ (mode = 6,000 versus 12,000 $\mu^2$ in controls), suggesting obliteration of a majority of glomerular tufts. Also, the hypertrophied glomeruli ($A_0 > 24,000 \mu^2$) observed after 12 months of CsA, were no longer evident once the injury had become decompensated (Figure 3). Presumably, hypertrophic remnant glomeruli are able to maintain two-kidney GFR constant during the compensated stage of injury. The passage to a decompensated stage likely reflects the inability of a dwindling number of remnant functional glomeruli to compensate for an ever-increasing fraction of glomeruli that has undergone obliteration.

**DISCUSSION**

Our findings and observations lead us to postulate the following hypothesis to explain the phenomenon of CICN. We submit that the primary injury is microvascular in nature. Both the physiologic and the morphologic findings point to an occlusive disorder affecting afferent arterioles. Ischemic damage to downstream nephrons includes the collapse and obliteration of affected glomerular tufts. The resulting loss of surface area for filtration serves to lower the GFR. The extent to which the GFR is lowered in a given individual appears to reflect an offsetting interplay between the fraction of glomeruli that have collapsed and the ability of spared, remnant glomeruli to undergo hypertrophy and increase their single nephron GFR (12). Adaptive growth and hyperfiltration by perfused, remnant glomeruli appear to offset a progressive increase in the fraction of obliterated glomeruli, thereby maintaining GFR constant for protracted periods of time. We have termed such adaptation as the "compensated stage" of the injury. Only when the dwindling fraction of spared glomeruli declines below some critical threshold does a "decompensated stage" become manifest. It is characterized by progressive azotemia, and ESRF eventuates.

Although this is based on indirect methodology, we estimate that afferent arteriolar resistance in cardiac Tx recipients could be elevated by as much as threefold after 12 months of CsA therapy in low dosage. Biopsy cross-sections are not adequate for assessing the fraction of afferent arterioles that is affected by an occlusive process. However, judged by a relatively low fraction of collapsed glomeruli at this time (Table 3), we surmise that the impingement on luminal diameter that attends the arteriolopathy is not solely responsible for the higher preglomerular RVR. Rather, there appears to be also a generalized increase in afferent arteriolar tone, with an ensuing fall in glomerular ultrafiltration pressure (9,10,13). Studies which employ acute or short-term administration of CsA to experimental animals have identi-
A disturbing feature of CICN is that it may continue to progress even after CsA is withdrawn (Figure 6). In this respect, it resembles other chronic renal diseases, in which a progressive course appears to be perpetuated even after the inciting etiologic agent is no longer present. As in other chronic renal diseases, the pathogenesis of progression is likely to be the same as that in postablation models of the remnant kidney in experimental animals (24,25). Examination of the serial biopsy findings in Table 3 reveals an incremental obliteration of patent, remnant glomeruli over a 24-month interval. Because small, collapsed glomeruli appear less frequently in a given cross-section than do larger patent glomeruli, it is likely that the actual increment in obliterated glomeruli was considerably greater than the apparent increment of 23%. From examination of the serial two-kidney values in Table 2, it is evident that single nephron GFR and the rate of transepithelial protein trafficking in individual surviving glomeruli must have increased in the interim. In addition to these functional hallmarks of the "remnant kidney" phenomenon, we have also demonstrated three of the morphologic hallmarks of this disorder, namely glomerular hypertrophy (Figure 4), an expanded mesangial matrix, and focal and segmental sclerosis (Table 3). We accordingly infer that a progressive course and evolution to ESRF in patients with CICN is a consequence of CsA-induced afferent occlusive arteriolopathy combined with the remnant kidney phenomenon.

Regardless of underlying etiology, most chronic renal injuries take between one and three decades to progress to ESRF. Given the associated stigma of the remnant kidney phenomenon, we believe that CICN is unlikely to be an exception to this rule. It follows that the cumulative incidence of ESRF in this disorder (approximately 10% in the first decade; Figure 5) can be expected to rise substantially with the passage of one or more additional decades. On the basis of this expectation, we propose that indefinite, continuous CsA therapy is not justified when the recipient is likely to survive for 10 yr or more. The latter criterion could be reasonably viewed as not applicable to allograft recipients, few of whom survive for more than 10 yr in the absence of CsA therapy. As a practical matter, therefore, it applies mainly to an evergrowing list of autoimmune diseases for which CsA has become a treatment of choice. We accordingly recommend that once autoimmune inflammation has been suppressed by a course of CsA of limited duration (<12 mo), alternative maintenance immunosuppressive therapy should be substituted.

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