A Randomized Double-Blind Placebo-Controlled Trial of Cyclosporine in Steroid-Resistant Idiopathic Focal Segmental Glomerulosclerosis in Children¹,²

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ABSTRACT

There is no generally accepted treatment for primary focal segmental glomerulosclerosis (FSGS). Steroids alone and steroids plus cyclophosphamide can be expected to induce a remission of the proteinuria in only 27% of patients. Probably the majority of FSGS patients will reach ESRD over the extended course of their disease. In addition to the work presented in this study, there have been many reports of the potential effectiveness of cyclosporine (CSA) in reducing the proteinuria of FSGS. This study was undertaken to test the efficacy and safety of a 6-month course of CSA in children with corticosteroid-resistant FSGS. The potential inhibitory effect of hypercholesterolemia on the proteinuria-reducing actions of CSA was also assessed. Twenty-five patients with FSGS were randomized to receive either placebo or CSA for 6 months. Twelve of the 12 patients that received CSA experienced a diminution of their proteinuria as opposed to only two of the 12 placebo-treated patients. Proteinuria was significantly reduced from 151.7 ± 162.4 mg/kg per 24 h at Week 0 to 36.9 ± 42.3 at the end of the study in the group that received CSA (P < 0.05). There was no significant change in the proteinuria of the patients in the placebo group. A significant correlation between the percentage change of proteinuria over the 6 months of the study and the pre-study serum cholesterol levels (r = 0.79, P < 0.05)

was seen in the CSA group. A partial correlation analysis controlling for the effects of serum cholesterol uncovered a significant relationship between average CSA level and proteinuria change (r = -0.76, P < 0.05). The fractional decline in GFR over the course of the study was not significantly different between the CSA and placebo-treated groups. In conclusion, CSA reduces proteinuria, increases serum albumin levels, and can be expected, therefore, to reduce the symptoms of nephrotic syndrome. Hypercholesterolemia antagonizes this effect of CSA.

Key Words: Nephrotic syndrome, hypercholesterolemia, cyclosporine, focal segmental glomerulosclerosis, proteinuria

Corticosteroid-resistant focal segmental glomerulosclerosis (FSGS) is the most common glomerular lesion leading to ESRD in children. Data from the transplant registry of the North American Pediatric Renal Transplant Cooperative Study (NAPRTCS) demonstrate that FSGS accounts for 12% of all pediatric renal transplants done in the United States and Canada (1). Additional data from the NAPRTCS dialysis registry show that FSGS also accounts for 12% of all children undergoing dialysis in the United States and Canada (2). There is no generally accepted treatment for the majority of patients with FSGS who are steroid resistant.

The introduction of cyclosporine (CSA) as an agent for the treatment of FSGS was predicated on two potential mechanisms. The specific T cell effects of this drug (3) could favorably affect the T cell dysfunction that has been postulated to be an important factor in the pathogenesis of proteinuria (4). Also, the CSA-induced preglomerular vasoconstriction (5) could result in a hemodynamically mediated proteinuria reduction. In our initial study, reported in 1986 (6), a remission of the nephrotic syndrome was induced in three of seven steroid-resistant FSGS patients with an 8-week course of CSA at a dose of 7 mg/kg per day. Since then, numerous uncontrolled studies (7-21) have used CSA with varying doses and duration in the treatment of steroid-resistant FSGS with mixed results.

This study was undertaken to test the efficacy and safety of a 6-month course of CSA in a double-blinded, prospectively randomized, placebo-controlled trial in children with steroid-resistant FSGS. Data analyses were performed to identify the factors that affect CSA efficacy. Hyperlipidemia is almost universally present in the nephrotic syndrome and is therefore an impor-
tant potential factor. The highly lipophilic nature of CSA could result in a decreased bioavailability of the drug (22-24). An inhibition of CSA effect by hypercho-
lesterolemia in patients with the nephrotic syndrome has been observed (25). CSA is known to be a chronic nephrotoxin (22), therefore, the effect of 6 months of CSA treatment on glomerular filtration was also evaluated.

METHODS

This study was performed with Food and Drug Adminis-
tration IND authorization (No. 30,667) and with the approval of the Institutional Review Boards at each of the participating centers.

Patients

To be included in the study, each patient had to satisfy the following criteria: (1) patients must be between 6 months and 21 yr of age; (2) patients had to have had a biopsy diagnosis of FSGS (confirmed by study pathologist) with significant proteinuria (greater than 4 mg/m² per h, random urine protein to creatinine ratio greater than or equal to 0.18 mg/mg in children or urine protein to creatinine ratio greater than or equal to 0.49 mg/mg in children below 2 yr of age) (26); (3) patients must have failed to fully respond (proteinuria declining into the normal range as defined in criterion 2) to a standard course of steroid therapy (prednisone 60 mg/m² daily in divided doses for 4 wk); (4) patients must have had a GFR of at least 40 mL/min per 1.73 m²; (5) sexually mature female patients must have had a negative pregnancy test at baseline and must procure acceptable birth control to be used throughout the duration of the study period; (6) patients with any recognized risk factors must have been tested for HIV, and; (7) written informed consent must have been given by the patient (or patient's guardian).

Patients were excluded from the study on the basis of the following criteria: CSA or other immunosuppressive therapy administered within 3 months of study entry; an identifiable primary etiology for FSGS lesions; concomitant therapy with a potentially nephrotoxic drug; use of an angiotensin-converting enzyme inhibitor; impaired liver function; inability to understand the protocol or attend regular outpatient clinic sessions; a significant concomitant disease or condition; or pregnancy.

Study Design

Patients were randomized at the time of study entry based on a previously computer-generated list of CSA- or placebo

suspension. Placebo patients received a vehicle control. Active drug and placebo suspensions were supplied by Sandoz Pharmaceuticals (Hanover, NJ). The unblinded clinical coordinator adjusted patients' study drug dose according to the following protocol. An initial dose of 0.03 mL/kg (3.0 mg/kg of CSA) twice daily was utilized with the objective of attaining a target CSA level within the range of 300 to 500 ng/mL. Dose reductions were triggered by a confirmed CSA level > 500 ng/mL, serum creatinine level > 0.3 mg/dL above baseline, SGOT > 90 IU/1, SGPT > 150 IU/1, or total bilirubin level > 2.25 mg/dL. Two progressive 0.01 mL/kg (1.0 mg/kg of CSA) per dose reductions, each observed for 2 wk, were utilized. If the condition still persisted, the dose was reduced a further 50% for an additional 2 wk. If the condition still persisted, the study drug was discontinued and the patient was dropped from the study. A CSA level of < 100 ng/mL triggered a dose increase of 1.5 mg/kg per dose. A CSA level of > 100 ng/mL but < 200 ng/mL triggered a dose increase of 1.0 mg/kg per dose. A CSA level of > 200 ng/mL but < 300 ng/mL triggered a dose increase of 0.5 mg/kg per dose. The failure to control hypertension with optimal dos-

ages of three antihypertensive agents from three modality categories was followed by successive study drug dose reduc-
tions, with potential patient removal from the study, as above. Each CSA-patient dose-adjustment notification (given in mL of study drug) was accompanied by a matched placebo-patient dose adjustment. Adverse reactions and the occurrence of known CSA toxicities were recorded.

Patients were monitored according to the following sched-
ule: vital signs, complete blood cell count, serum chemis-
tries, urinalysis, quantitative proteinuria, and CSA level weekly for the first 4 wk, then monthly. The first set of monitored values, reported as "pre", were obtained at Week 0 (i.e., before the first dose of the study drug). Values reported as being obtained at the "end" of the study were acquired at week 24 (with the patient still receiving the study drug). Values reported as "post" were obtained at week 28 (i.e., 1 month after the patient discontinued the study drug). GFR were determined before, beginning, and after discontinuing the study drug. The study drug could be temporarily with-
held on the basis of either an intercurrent infection or contact with varicella.

The following medication stop points were employed: (1) after 6 months of treatment; (2) presence of a potentially serious infection; (3) persistent elevation of creatinine, potas-
sium, bilirubin, liver enzymes, or blood pressure levels; (4) development of a malignancy (including lymphoproliferative disorders); (5) development of an independent disease requiring treatment not permissible in the protocol, as defined by the inclusion and exclusion criteria; (6) at the request of the patient (or guardian); (7) occurrence of other significant adverse effects not resolved by the application of the above dose reduction scheme; (8) poor compliance with the study regimen; (9) pregnancy, and (10) at the discretion of the investigator. During the course of study treatment, the following were strictly contraindicated: other immunosuppres-
sive agents, angiotensin-converting enzyme inhibitors, and plasmapheresis. Treatment with potentially nephrotoxic drugs and drugs known to interact with CSA were to be avoided. Calcium channel blocking agents were recom-
mended for the treatment of hypertension.

The study was not designed to test the efficacy of CSA therapy beyond the 6-month treatment period. Participating centers were authorized to choose either "center specific" therapy or to continue treatment with CSA.

Testing

Serum and urine biochemical parameters were determined by standard laboratory methods. CSA was initially monitored as trough, whole blood levels by polyclonal RIA. The study reference laboratory (MetPath) changed their methodology to specific monoclonal-based assay approximately midway through the study. Proteinuria was assessed either through a 24-h urine collection or the determination of the protein to creatinine ratio in an early morning sample urine. Protein-creatinine ratios were converted to the equivalent 24-h protein excretion value (27). GFR was determined with Technetium-99m-DPTA as the filtration marker, utilizing a two-plasma sample method for calculation (28).
Data Analysis

Patients were judged to have had no response to treatment if their proteinuria did not decline during the course of the study. Patients whose proteinuria declined into the normal range (as defined in "Patients," Study Inclusion Criterion 2, above) during the study period were recognized as having entered a complete remission. A partial response was defined as a reduction in proteinuria, but still remaining in the supranormal range. The total improved in each group was defined as the sum of those patients who experienced a complete remission or a partial response. Data was analyzed on a per-protocol basis.

Statistical analysis was performed utilizing t test, chi-square testing, partial correlation analysis, and multiple regression analysis. All data are expressed as the mean ± SD. Significance was considered to be \( P < 0.05 \). Graphs were produced with SigmaPlot for Windows (Jandel Scientific, San Francisco, CA) and statistical analyses were performed with SPSS for Windows (SPSS Inc., Andover, MA).

RESULTS

Eight centers submitted biopsy specimens from 39 patients for review. One specimen was found not to be consistent with FSGS by the central study pathologist. Seven patients either failed to satisfy the inclusion criteria or met an exclusion criterion. Thirty-one patients were available for randomization. Before randomization, informed consent was obtained. Sixteen patients were assigned to receive CSA and 15 patients received a placebo control. Two patients in each group were withdrawn because of noncompliance with the study protocol. One CSA patient requested withdrawal with no specific reason given. One patient from each group was withdrawn for a progressively rising serum creatinine level not responsive to the protocol-dictated study drug-dose reductions. Twelve CSA patients and 12 placebo patients completed the full 6-month study course. There were no significant differences between the CSA and placebo groups in: male to female ratio, age, time from renal biopsy that was diagnostic of FSGS to study entry, initial GFR (by nuclear disappearance methodology), prevalence of hypertension (defined as the need for antihypertensive medication), and initial proteinuria (Table 1). The initial serum albumin and cholesterol values were not significantly different between the study groups (Table 2).

Categorization of the patients by clinical response is enumerated in Table 3. Four CSA patients entered a complete remission (as compared with none of the placebo patients, \( P < 0.05 \)); and eight CSA patients had a partial response (as compared with two placebo patients, \( P < 0.05 \)). All of the patients receiving CSA demonstrated at least some improvement (i.e., diminution of proteinuria).

Proteinuria was significantly reduced from 151.7 ± 162.4 mg/kg per 24 h at Week 0 to 36.9 ± 42.3 at the end of the study in the group that received CSA (\( P < 0.05 \)), as demonstrated in Figure 1. There was no significant change in the proteinuria of the patients in the placebo group (pre: 166.9 ± 137.1 to end: 195.4 ± 173.7, \( P = \text{not significant} \) [NS]). Individual patient proteinuria changes by group are presented in Figure 2. Proteinuria in the CSA group declined by 70.7 ± 19.2% as compared with an increase of 11.4 ± 29.0% in the placebo group (\( P < 0.05 \)). The time to response (at least a 50% reduction in proteinuria) for the CSA-treated patients was 4.4 ± 1.8 wk.

When factored by GFR (calculated from a contemporaneous serum creatinine level [29]), CSA-group proteinuria still significantly declined from 6.0 ± 7.5 mg/100 mL glomerular filtrate to 1.7 ± 2.0 over the course of the study (\( P < 0.05 \)). Placebo-group proteinuria remained not significantly changed when expressed as mg per 100 mL of glomerular filtrate (pre: 5.6 ± 4.4 to end: 9.6 ± 11.3, \( P = \text{NS} \)). The difference between the two groups of the percentage changes of proteinuria per 100 mL GF was highly significant (CSA: −60.6 ± 37.7%, placebo: 63.5 ± 128%, \( P < 0.005 \)).

The changes in serum biochemical values are presented in Table 3. Serum albumin, creatinine, and potassium levels rose significantly in the CSA group; serum magnesium levels declined. Indices of liver function and total cholesterol levels remained unchanged in this group. There were no significant changes in any of these values in the placebo group.

| TABLE 1. Characteristics of the patient groups at the time of randomizationa |
|-----------------|--------|--------|
| Characteristic   | CSA    | Placebo|
| \( N \)          | 15     | 15     |
| Male/Female      | 11/4   | 10/5   |
| Age (range, in yr) | 11.2 ± 4.2 (2-18) | 11.4 ± 3.9 (3-19) |
| Time from Diagnostic Biopsy (range, in yr) | 0.8 ± 0.7 (0.3-2.2) | 1.7 ± 2.2 (0.3-6) |
| Hypertensive (number) | 6      | 5      |
| Initial GFR (range, in mL/min per 1.73 m²) | 103.4 ± 36.7 (57.6-171.2) | 86.0 ± 31.3 (51.1-150.8) |
| Initial Proteinuria (range, in mg/kg per 24 h) | 151.7 ± 162.4 (11.1-566.2) | 166.9 ± 137.1 (38.1-364.5) |

\( a \) \( P = \text{NS} \), for all values.
The GFR of the groups obtained before the initiation and after the discontinuation of the study drug were measured by DTPA clearance. Both the "pre" and "post" clearance studies were technically acceptable in eight patients in the CSA group and nine patients in the placebo group. GFR marginally, but significantly, declined over the course of the study from 103.4 ± 36.7 to 82.9 ± 19.1 in the CSA group (P = 0.05); and insignificantly declined from 86.0 ± 31.3 to 75.1 ± 30.6 in the placebo group (P = 0.06) (Figure 4). However, the fractional decline in GFR (expressed as the percentage change of the poststudy value from the presudy value) was not significantly different between the two groups: -15.7 ± 18.4% in the CSA group, and -11.8 ± 19.0% in the placebo group (P = NS).

The study was not designed to evaluate the long-term efficacy of CSA. At the end of the study period, participating centers were free to choose "center specific best therapy" for their patients. Of the patients who did not continue further CSA therapy, seven reached ESRD within 1 to 4 yr (four placebo, three CSA), and four are approaching ESRD status (two placebo, two CSA). However, ten patients (five placebo,
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In the CSA group developed mild gingival hyperplasia; two patients in each of the study groups had tercurrent infection; one patient in the CSA arm had the study drug temporarily suspended during an infection. Five CSA) are being maintained on continued CSA therapy since the end of the study period. These ten patients have remained in remission and are being maintained on CSA in doses ranging from 6 to 12 mg/kg per day with stable renal function.

The following adverse events were reported: two patients in the CSA group developed mild gingival hyperplasia; two patients in each of the study groups developed a worsening of hypertension that necessitated the initiation of additional antihypertensive agents; two patients in each of the study groups had the study drug temporarily suspended during an intercurrent infection; and one patient in the CSA group had the drug briefly withheld after a varicella exposure.

DISCUSSION

There is no generally accepted treatment for primary FSGS. A minority of the patients are steroid responsive (30). In the only controlled study, both steroids alone and steroids plus cyclophosphamide induced a remission of the proteinuria in only 27% of the study subjects (31). Nonsteroidal anti-inflammatory drugs have been reported to reduce the proteinuria in this condition, but the use of these agents is limited by their inconsistent efficacy and renal toxicity (32). At least 50% of FSGS patients with heavy proteinuria progress to ESRD over 7 to 10 yr (30). Probably the majority of FSGS patients will reach ESRD over the extended course of their disease. The recent report of Mendoza et al. (33) demonstrating a high rate of remission of proteinuria, with a diminished likelihood of progression to ESRD, with an aggressive regimen of high dose steroids and cyclophosphamide has not been confirmed by others (34,35). In addition to our own work (6), there have been many reports of the potential effectiveness of CSA on reducing the proteinuria of FSGS (7–21). However, this study is the first double-blinded, randomized, placebo-controlled trial of CSA in FSGS.

Although the pathogenesis of nephrotic syndrome, in general, and FSGS, in particular, are unknown, there are two potential mechanisms for a beneficial effect of CSA. In 1974, Shaloub suggested that the altered glomerular permeability of nephrotic syndrome was a reflection of changes in T cell function (4) and CSA is an agent that specifically affects T cell function (3). The transcription of mRNA for the immunoregulatory cytokine interleukin-2, a key signal for lymphocyte activation, is inhibited by CSA (3). We have previously noted that interleukin-2 activity increased in the supernatant of stimulated peripheral blood mononuclear cells of some patients with nephrotic syndrome (36). Because the half life of interleukin-2 in plasma is very brief (37) and IL-2 activity can only be demonstrated in cell suspension supernatant after PHA or mitogen stimulation, recent studies of nephrotic syndrome patients have measured the soluble receptor of IL-2 (released into the circulation by activated T cells) (38). Serum IL-2R levels have been reported to be elevated in patients with nephrotic syndrome, including Minimal Change Disease and FSGS but not congenital nephrotic syndrome, and to correlate with disease activity (39–41). To the extent that T cell activation promotes proteinuria, CSA would ameliorate the nephrotic syndrome by inhibiting the IL-2 driven amplification pathway.

Also, because CSA is known to cause preglomerular vasoconstriction (5), a reduction in filtration through a decrement in glomerular plasma flow or ultrafiltration pressure could reduce proteinuria on a purely hemodynamic basis. Alterations in the other main determinant of single-nephron GFR glomerular barrier function, have been demonstrated. Studies performed by Zieste and associates have shown that CSA reduces the fractional excretion of protein in patients with nephrotic syndrome (42) by decreasing the product of effective hydraulic permeability and glomerular capillary surface area (43).

The decline in proteinuria experienced by every patient in the CSA arm of our study was not simply the result of changes in GFR. When proteinuria was fac-
tored by GFR and expressed as quantity per glomerular filtrate. There still was a significant decline in the mean proteinuria of the CSA group. In this study, GFR was measured by a previously validated radionuclide clearance technique. To avoid confusion with the well-described acute nephrotoxicity of CSA, the second GFR value for each patient was obtained after the patient had been off the study drug for at least 2 wk. Both the CSA- and placebo-treated patient groups demonstrated a decrease in GFR from the prestudy to the poststudy value. However, there was no significant difference between the percentage GFR changes of the two groups. We therefore postulate that the decreased GFR in both arms of the study reflect the natural course of steroid-resistant FSGS. In this context, it is interesting to note that children usually exhibit less nephrotoxicity as a result of CSA than adults (48) and that GFR measured in a group of children receiving long-term CSA remained stable after an initial drop (49). Both of the patients who were withdrawn from the study for increasing serum creatinine levels continued to have rising creatinine levels after leaving the study (only one had received CSA), consistent with the progression of FSGS.

In summary, in this controlled trial, CSA administered for 6 months produced an amelioration of proteinuria in the study group without undue toxic effects. We conclude that for steroid-resistant patients with FSGS, CSA is an additional therapeutic approach for a disease with limited options and a virtually certain progression to ESRD. A specific indication for the use of CSA would be for the control of the symptoms of resistant nephrotic syndrome (most commonly, edema). Future studies further optimizing the regimen for the administration of CSA (increased dose with hypercholesterolemia and longer duration) might eventually provide an effective treatment that improves the generally poor prognosis of FSGS.

APPENDIX

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