Severe Hypercholesterolemia Inhibits Cyclosporin A Efficacy in a Dose-Dependent Manner in Children With Nephrotic Syndrome

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ABSTRACT

In order to identify possible markers of cyclosporin A (CSA) efficacy, the use of CSA (6 mg/kg) in 47 children with refractory nephrotic syndrome was reviewed. Response was defined as remission of proteinuria within 2 months. Before CSA administration, nonresponders (N = 13) were found to have more proteinuria (6 versus 3 g/24 h; \( P < 0.03 \)) and higher serum creatinine levels (0.9 versus 0.6 mg/dL; \( P < 0.03 \)) compared with responders (N = 34). Also, a markedly elevated serum cholesterol level (545 versus 312 mg/dL; \( P < 0.001 \)) was noted among nonresponders. Logistic regression analysis of all three parameters isolated serum cholesterol (\( P < 0.01 \)) as the only significant predictor of CSA nonresponsiveness. Discriminate analysis identified serum cholesterol to predict 97% of responders and 77% of nonresponders (\( P < 0.0005 \)) to conventional CSA doses. The CSA whole-blood trough HPLC levels were subtherapeutic among nonresponders (71 ng/mL) compared with responders (162 ng/mL) (\( P < 0.001 \)). Thus, a high serum cholesterol level may prevent the achievement of therapeutic CSA blood levels with conventional doses. On the basis of this, seven of the nonresponders were re-treated by titration of the CSA dose (10 to 14 mg/kg) with their serum cholesterol level. Their mean highest trough CSA level was 286 ng/mL. Five patients responded within 2 months. No elevation in serum creatinine or evidence of nephrotoxicity on repeat biopsy was seen after 2 months of therapy in all seven patients. It was concluded that severe hypercholesterolemia in nephrotic syndrome patients necessitates the titration of the CSA dose with the serum cholesterol level to achieve remission.

Key Words: Nephrotic syndrome, pediatric, cyclosporin A, hypercholesterolemia

Persistent proteinuria in patients with idiopathic nephrotic syndrome (NS) has been correlated with a gradual loss of renal function (1). In an attempt to halt the progression of disease, numerous therapies have been used with mixed results. Efforts to control the proteinuria are based on evidence to support T cell dysfunction as a mediator of disease (2–4). Corticosteroids have been the mainstay of therapy for NS. Steroids have been shown to block the release of interleukin-1 (IL-1) by mononuclear cells (5). Chemotherapeutic agents, such as cyclophosphamide and chlorambucil, are T cell cytolytic and alter T cell memory (6). The ravages of steroid toxicity (7) and the dose-dependent gonadal dysfunction induced by cyclophosphamide and chlorambucil (8) have prompted the search for alternative therapies.

Cyclosporin A (CSA), a fungal decapeptide, has been shown to block the production and release of cytokines, specifically IL-2 (9, 10). Trials with this immunosuppressive agent in NS patients with a variety of histologic lesions failed to demonstrate uniform efficacy (11, 12). In an attempt to identify factors that may affect CSA efficacy, we have reviewed a large cohort of children with NS treated with CSA at SUNY-Health Science Center at Brooklyn.

MATERIALS AND METHODS

Phase 1

The study was carried out in two phases. In order for NS patients to be included in phase 1 of this study (1984 to 1990), they had to be either steroid dependent or steroid resistant. Steroid dependence was defined as a remission/reduction in proteinuria with the introduction of prednisone and a relapse upon its tapering or withdrawal. Patients whose proteinuria was unaffected by steroid therapy were designated steroid resistant. Additionally, all patients had to
demonstrate active disease (i.e., NS) at the time of CSA administration. Informed consent was obtained, and a percutaneous renal biopsy was performed. Each patient’s serum albumin, creatinine, cholesterol, and proteinuria were quantitated the morning before the initiation of CSA. The methodology of these determinations has been described (13).

Patients were monitored weekly in the renal clinic. At that time, a urine analysis was carried out with chemstrip dipsticks (Miles Diagnostic, Elkhart, IL). A quantitative urine protein determination was made twice monthly. Serum albumin, creatinine, and cholesterol levels were monitored weekly. CSA was started at a dose of 6 mg/kg/day given in two equally divided oral doses. CSA whole-blood trough levels were measured weekly by HPLC. The dose was lowered for a level >300 ng/mL but was not increased for a level <100 ng/mL. The dose of CSA was also lowered for an increase in the patient’s serum creatinine ≥0.3 mg/dL above baseline on two consecutive determinations. The dose was not increased with lack of response. After 8 wk of CSA therapy, the drug was abruptly discontinued and the data were analyzed. Response was defined as remission of proteinuria (≤4 mg/m²/h).

Phase 2

On the basis of the data analysis in phase 1 of this study, nonresponders with normal renal function and persistent NS were entered into phase 2 of the study (1989 to 1991). Patients were initially retreated with 10 mg/kg/day of CSA. In the face of severe hypercholesterolemia (>300 mg/dL), the dose of CSA was increased in a stepwise manner to a maximum of 14 mg/kg/day until a decrease in the proteinuria or serum cholesterol level was noted. The CSA whole-blood trough and serum creatinine levels were monitored twice weekly. As the serum cholesterol levels decreased during treatment, the CSA dose was slowly reduced. As in phase 1, CSA was administered for a total of 8 wk and was abruptly discontinued. Repeat percutaneous renal biopsies were performed upon the completion of phase 2.

Histology

Nephrotoxicity was diagnosed by the presence of tubular atrophy, stripe interstitial fibrosis, and arteriolopathy on biopsy specimens (14). Minimal-change disease was defined as normal glomeruli seen by light microscopy, negative results on immunofluorescence, and the effacement of foot processes of podocytes by electron microscopic examination (15). The diagnosis of mesangial hypercellularity was made when three or more mesangial cells were seen in a mesangial stalk away from the hilum in at least 10% of glomeruli on light microscopic examination; con-

firmation was by electron microscopic examination (15). Focal segmental glomerulosclerosis (FSGS) was defined as segmental sclerosis and hyalinosis of one or more glomeruli on light microscopic examination, together with corresponding deposition of immunoglobulin M (IgM) and C3 detected by immunofluorescence and obliteration of capillary lumina and capillary-wall collapse by electron microscopic examination (15). IgM nephropathy was defined as the mesangial deposition of IgM together with mesangial and/or paramesangial electron-dense deposits (16).

Statistical Analysis

Statistical analysis was carried out by t test comparing mean values and a χ² analysis comparing individual values, as well as values between groups. In addition, a logistic regression analysis and a discriminate analysis were carried out to identify factors leading to nonresponsiveness. A P value <0.05 was considered significant.

RESULTS

Forty-seven children (31 boys and 16 girls) met criteria for entry into phase I of this study. The mean age was 5.4 ± 4.0 yr. Racial distribution included 26 black, 9 Hispanic, and 7 white patients and 5 patients of other descent. Twenty-six were steroid dependent, 20 were steroid resistant, and 1 was not treated with steroids. Results of the biopsies before entry into phase I established FSGS as the predominant histologic lesion (N = 24). Other lesions included IgM nephropathy (N = 12), minimal-change disease (N = 8), and mesangial hypercellularity (N = 3).

Phase 1

At the end of phase 1, 34 patients (72%) responded to CSA therapy. Twenty-five (73.5%) of the 34 responders were steroid dependent, and 8 (23.5%) were steroid resistant. Almost all of the patients that initially did not respond to CSA therapy were steroid resistant (12 of 13). The histology of patients who responded compared with those who did not is depicted in Figure 1. Thirteen (54%) of the 24 patients with FSGS responded to conventional CSA doses; thus, the histologic lesion of FSGS did not predict nonresponse. Nonresponders were found to have more proteinuria compared with responders (6.0 ± 4.5 versus 3.1 ± 1.9 g/24 h; P < 0.03); however, the mean serum albumin was similar (2.1 ± 0.9 versus 2.0 ± 0.5 g/dL; P = not significant). Although the mean serum creatinine was higher among nonresponders (0.9 ± 0.4 versus 0.6 ± 0.3 mg/dL; P < 0.03), the duration of NS before CSA was started was not different (2.2 ± 1.7 versus 3.8 ± 4.2 yr). The most
striking finding noted was a markedly elevated mean serum cholesterol level (545 ± 206 versus 312 ± 76 mg/dL; \( P < 0.001 \)) in nonresponders compared with responders (Figure 2). The mean CSA blood level was subtherapeutic among nonresponders (71 ± 14 ng/mL) compared with responders (162 ± 40 ng/mL) \( (P < 0.001) \).

Because serum cholesterol, creatinine, and proteinuria were all found to be significantly different among the two groups, a logistic regression analysis was carried out to identify which of the three parameters, if any, were of independent significance. Regression analysis isolated serum cholesterol as the only significant predictor of CSA nonresponsiveness \( (P < 0.01) \). A discriminate analysis was carried out to determine if any of the three parameters could predict CSA efficacy. The serum cholesterol level was found to predict 97% of those patients who would respond to conventional CSA doses and 77% of those who would not \( (P < 0.0005) \).

Because FSGS patients comprise 84% of nonresponders, an additional analysis was carried out to assess if patients with FSGS had a higher serum cholesterol level and thus would predictably not respond. The mean serum cholesterol level of the 13 FSGS responders (316 ± 93 mg/dL) was not different from the mean serum cholesterol level of the 21 non-FSGS responders (306 ± 63 mg/dL). However, the mean serum cholesterol level of the 11 FSGS nonresponders (561 ± 221 mg/dL) was significantly higher when compared with the mean serum cholesterol levels of the 13 FSGS responders \( (P < 0.001) \) and the 21 non-FSGS responders \( (P < 0.001) \). Thus, the histologic lesion observed did not predict higher serum cholesterol levels.

In order to determine if disease severity (i.e., number of relapses) would predict hypercholesterolemia, we divided the patients who responded to CSA into three groups and compared the mean serum cholesterol levels drawn before CSA therapy between them. Patients with three relapses had a mean serum cholesterol level of 323 ± 61 mg/dL. Patients with 4 to 12 relapses of their NS had a mean serum cholesterol level of 320 ± 84 mg/dL, and patients who never responded to steroid therapy and thus were nephrotic throughout the course of their disease had a mean serum cholesterol level of 300 ± 97 mg/dL. These values were not different from each other, and thus, disease severity did not predict hypercholesterolemia.

Phase 2

On the basis of the correlation between markedly elevated serum cholesterol levels and the CSA nonresponsiveness found in phase 1, 7 of the 13 nonresponders were re-treated with CSA in phase 2. Two of the remaining six patients not re-treated had reached ESRD, two had elevated serum creatinine levels (>2 mg/dL), one patient refused further therapy, and one patient was incarcerated in a penal institution and was unable to be closely monitored.
The seven re-treated patients received CSA at a high dose between 10 and 14 mg/kg/day. During the 8 wk of therapy, their mean highest trough CSA level was 286 ± 80 ng/mL. Five of the seven patients responded within 2 months of higher dose therapy. The mean serum creatinine (Figure 3) in all seven patients at the completion of phase 2 was identical to that at the onset of phase 1 (0.9 ± 0.4 mg/dL). No evidence of nephrotoxicity was seen on repeat biopsy in any of the seven patients.

Outcome

Of the 34 patients who responded to CSA therapy after phase 1 of the study, 11 patients demonstrated a sustained remission less than 3 months after the withdrawal of CSA therapy, 8 patients did so between 3 and 6 months, 6 patients did so between 6 and 9 months, 5 patients did so between 9 and 12 months, and 4 patients continued to be in remission of their NS for more than 12 months. All five of the patients re-treated with higher doses of CSA in phase 2 of the study relapsed within 3 months of CSA withdrawal. Three of the five patients were re-treated with CSA. A remission was once again achieved at doses between 8 and 9 mg/kg/day. Maintenance CSA at doses of 5 to 6 mg/kg/day is necessary to continue remission in these three patients.

DISCUSSION

The failure of corticosteroids and alkylating agents in ameliorating the proteinuria in refractory NS patients has prompted investigations for alternative therapies. Nonsteroidal anti-inflammatory drugs (17) have been shown to reduce the proteinuria in NS by decreasing the GFR. On the basis of the propensity of CSA to decrease RPF, it has been suggested that the CSA-induced remission of NS is mediated by decreasing the GFR (18). Recent studies by Zeiste and colleagues (19, 20) have demonstrated that CSA decreases proteinuria through increasing the permselectivity of the glomerular basement membrane and not by decreasing GFR. Studies of the nephrotic mouse model treated with CSA (21) substantiate the observations of Zeiste et al.

Current knowledge implicates cytokines, T cell mediators, as the primary offenders in disrupting the permeability of the glomerular basement membrane, thus precipitating the proteinuria of the NS (22). Elevated levels of IL-2, a cytokine produced by T-helper cells, have been demonstrated in patients with refractory NS (23). Upon treatment with CSA, the decrease in IL-2 preceded a remission of proteinuria and the increase in the IL-2 level after the withdrawal of CSA heralded a relapse (24). Clinical trials with CSA in NS patients have demonstrated a response rate of 40 to 60% (25). The standard dose of CSA is 5 to 6 mg/kg/day. Our study demonstrates that conventional doses of CSA fail in almost 30% of our patient population; however, higher doses of CSA induced a remission in some of these patients. CSA nonresponsiveness was correlated with severe hypercholesterolemia. This may be explained by CSA distribution in the blood and entry into target tissues.

CSA is a highly lipophilic drug. Forty to 60% of the drug is bound to both red and white blood cells, 40 to 60% is bound to lipoproteins (high and low density), and less than 5% is present as free drug in the plasma (26). In a patient with type 5 hyperlipoproteinemia, it has been shown that the CSA bound to cholesterol in the blood was not biologically active (27). In patients with elevated serum cholesterol levels, more CSA is bound in the lipoprotein compartment, thus decreasing the amount of available drug (28, 29). deGroen (30) has hypothesized that CSA enters the cell by binding to a low-density lipoprotein receptor complex and is incorporated in the cell by receptor-mediated endocytosis. Once inside the cell, it binds to a cytosolic receptor, cyclophilin, which is translocated into the nucleus where it inhibits the proliferation and activation of T-helper cells by altering production and response to IL-2. The hypercholesterolemia is thought to inhibit entry into the cell by causing a down-regulation of the receptor complex on target tissue (31).

In a previous report, we have demonstrated the
efficacy of high-dose CSA therapy in successfully shutting off recurrent proteinuria posttransplantation in the face of hypercholesterolemia without nephrotoxicity (31). Morales et al. have also noted similar findings (32). Nephrotoxicity associated with CSA administration in the transplant population has been correlated with age, high-trough CSA blood level (33), and prolonged duration of therapy (34). Children are known to tolerate higher doses of CSA because of their higher metabolic rates (33). Unlike data from a recent study in adults (22), long-term studies in children with steroid-resistant FSGS receiving CSA for 18 to 26 months failed to demonstrate nephrotoxicity (35, 36). We suggest that the absence of CSA-induced nephrotoxicity in our patients is based on the age of our patient population, the low-trough CSA blood levels, and the short duration of therapy (2 months). Additionally, we suggest that the hypercholesterolemia observed in these patients may be protective against CSA-induced toxicity because others have noted an absence of nephrotoxicity in bone marrow transplant recipients with higher serum cholesterol levels despite high-trough CSA blood levels (37). In conclusion, severe hypercholesterolemia in NS patients necessitates the titration of the CSA dose with the serum cholesterol level in order to achieve remission without incurring nephrotoxicity.

REFERENCES


