

Cyclosporin in steroid-resistant nephrotic syndrome

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Cyclosporin A (CyA) has recently been shown to be effective in frequently relapsing, steroid-resistant or steroid-dependent childhood nephrotic syndrome (NS). Wider acceptance has been hindered by considerations of cost, toxicity and drug-dependent maintenance of remission. Black children in Africa with NS are mainly steroid-unresponsive and alternative therapies therefore need to be assessed.

We report on 8 of 11 children who completed at least 24 weeks of CyA therapy; 6 (2 with membranous nephropathy (MEM) and 4 with focal glomerulosclerosis) showed no improvement, while 2, both with MEM, achieved remission while on CyA therapy and remained in remission for 1 and 3,5 years, respectively, after cessation of therapy. This may have been the natural course of the disease. Of the children who were unresponsive to CyA, 3 died in renal failure 8 - 30 months after cessation of CyA therapy, 1 had a rising creatinine value when last seen, and 2 were lost to follow-up. CyA trough levels varied between 180 and 875 ng/ml and peak levels between 563 and 1 950 ng/ml. Of 5 repeat renal biopsies, 3 were performed at the end of 24 weeks of treatment and revealed no evidence of CyA toxicity. Two biopsies revealed chronic CyA toxicity. CyA should therefore be used with caution at lower dosages and monitored at currently accepted lower trough levels in children with NS in Africa.

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Cyclosporin A (CyA) is the mainstay of immunosuppressive therapy in all transplant centres and has been used more recently in auto-immune disease^{1,2} and in nephrotic syndrome (NS). It has been shown to be useful in steroid-resistant, steroid-dependent³ and steroid-responsive frequently relapsing NS, and in patients with steroid toxicity.⁴ Major disadvantages of CyA are nephrotoxicity and relapse on cessation of therapy.⁴ There is no suitable or effective therapy for the black child with NS in South Africa, because these patients are usually steroid-insensitive.^{5,6} Among Indian South Africans the pattern of NS is similar to that in white children and focal glomerulosclerosis (FGS) therefore poses a major therapeutic challenge.

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In contrast to the present situation, when we first considered the use of CyA for NS in 1984, little information was available on the use of this drug in children, particularly in blacks. Accordingly, a study was embarked upon to determine suitable dosages, duration of therapy, toxic effects and the likely therapeutic advantages of CyA in nephrotic children who were steroid-resistant or for whom steroids were contraindicated (i.e. those with MEM).

Patients and methods

Study design

Study criteria

Selection criteria for entry to the study included oedema, hypo-albuminaemia (< 30 g/l), massive proteinuria (> 2 g/m²/d), any black or Indian child with FGS, and membranous nephropathy (MEM), or minimal change disease (MCD) in blacks. Criteria for exclusion were acute or chronic viral, fungal or bacterial (including tuberculosis) infections, impaired hepatic, renal or neurological function, concomitant therapy with nephrotoxic agents, cytostatics, ketoconazole, phenytoin, barbiturates, rifampicin, isoniazid or steroids, malabsorption, cancer, end-stage irreversible renal disease, diabetes mellitus, uncontrolled hypertension and drug or alcohol abuse.

Clinical evaluation

During the trial patients were evaluated clinically for signs and symptoms of NS and for side-effects of CyA. They were monitored for hepatic dysfunction (serum bilirubin value > 17 mmol/l, 3-fold increase in aspartate aminotransferase (AST)), renal dysfunction (serum creatinine value > 160 µmol, hypertension), neurological dysfunction (tremor, hyperparaesthesiae, convulsions), and general effects such as hirsutism and gum hypertrophy.

Laboratory investigations

Laboratory investigations included a full blood count and measurement of blood urea, electrolytes and creatinine values, serum albumin, globulin, calcium, phosphorus, bilirubin, alkaline phosphatase, AST and gamma-glutamyl transpeptidase; the urine was tested for blood, bilirubin, urobilin and glucose. Clinical evaluation and laboratory investigations were performed just before commencing CyA therapy, weekly for 4 weeks, every 2 weeks for 16 weeks, then every 4 weeks until 32 weeks. T-lymphocyte subpopulations were determined using the OKt series of murine monoclonal antibodies (Ortho Pharmaceuticals Corporation, Raritan, NJ). These data have been reported⁷ and are therefore not included in this study. These tests were undertaken before commencement of CyA therapy and 24 weeks later.

Cyclosporin dosage

In order to establish the dosage of CyA to achieve adequate whole-blood trough levels, CyA was commenced at 2,5 mg/kg/d in 2 divided doses and increased to achieve trough levels of 250 - 1 000 ng/ml. CyA was measured by radio-immunoassay.⁸ The course was given for 24 weeks.

Patients were withdrawn from the study if non-compliant or if any of the signs of toxicity occurred. Renal biopsy was repeated, if possible, after 24 weeks of therapy.

Patient details

On the basis of the above criteria 11 patients with NS (10 black and 1 Indian) were studied; their ages ranged from 2 to 14 years, and 5 were males. The duration of NS before commencing CyA therapy ranged from 6 to 39 months. Histological findings at the start of the project indicated that 5 patients had hepatitis B surface antigen (HBsAg)-associated MEM, 3 FGS (1 Indian), 2 MCD and 1 proliferative nephropathy. One of the 2 black children classified as having FGS had evidence of MCD on the first biopsy but was found to have FGS on repeat biopsy 13 months after cyclosporin therapy (referred to as MCD/FGS). All the children were given diuretics and a salt-restricted diet. Three of the black patients (2 MCD, 1 FGS) were given steroids without response. The Indian child with FGS was given both steroids and cyclophosphamide, with little effect.

Results

Clinical course and outcome (Table 1)

Eight of the 11 patients completed at least 24 weeks of CyA therapy; 4 of these 8 continued to 28, 32, 32 and 36 weeks, respectively. Of the 3 children who did not complete 24 weeks of treatment, 1 with MEM developed massive haematuria at 6 weeks and careful investigation ascribed the cause of the haematuria to cystitis unrelated to CyA. A patient with proliferative nephropathy was withdrawn by the parents at 7 weeks, and another with MCD defaulted after completing 12 weeks of treatment.

Table 1. Cyclosporin treatment — patient details

Patient	Age (yrs)	Sex	Histology	Trough levels (ng/ml)	Outcome
1	7	F	MEM*	210 - 250	Remission
2	6	M	MEM*	170 - 300	Remission
3	7	M	MEM	320 - 350	No change
4	11	M	MEM	220 - 390	No change
5	9	F	FGS*	270 - 330	No change
6	3,5	F	FGS	180 - 280	No change. Death 30 mo. after CyA. ESK. CT
7	14	M	FGS	450 - 875	No change. Death 10 mo. after CyA
8	2	F	MCD/FGS	240 - 280	Chronic renal failure, tubular rickets, 8 mo. after CyA therapy. CT

*No acute CyA toxicity after 24 wks of CyA.
ESK = end-stage kidney 10 mo. after CyA; CT = chronic CyA toxicity.

At the end of their course of treatment 6 patients (2 MEM, 3 FGS, 1 MCD/FGS) showed no change in their NS. Two patients, both with MEM, achieved remission while on CyA; in 1 of these patients the HBsAg and hepatitis B e antigen cleared progressively during the course of therapy. The serum creatinine levels in these 2 patients remained normal throughout the course of therapy and they continued in remission for 1 and 3,5 years, respectively, after cessation of CyA therapy.

The subsequent course in 3 of the 6 patients whose NS was unresponsive to CyA is as follows. The patient with FGS died 2,5 years after cessation of CyA therapy; she had not attended follow-up visits for 2 years because of family difficulties. The baseline serum creatinine value was 35 mmol/l and 4 months after withdrawal of CyA it was 60 mmol/l. She was admitted in renal failure, with a blood pressure of 144/100 mmHg, a haemoglobin concentration of 6 g/dl, a blood urea value of 54 mmol/l, and a serum creatinine value of 1 128 mmol/l. Despite dialysis and transfusion she died 24 hours after admission. The child with MCD/FGS, who had had CyA for 32 weeks, presented 8 months later unable to walk and with painful knees. Rickets was diagnosed. At this time investigations revealed the following: blood urea 12,6 mmol/l, serum creatinine 106 mmol/l, serum calcium 1,5 mmol/l, serum phosphate 0,75 mmol/l, alkaline phosphatase 1 329 U/l, phosphate excretion index 0,22% (normal -0,09 - +0,09%), and 25-hydroxyvitamin D 5 ng/ml (< 10 ng/ml indicates vitamin D deficiency). She had metabolic acidosis and panamino-aciduria. It was believed that she had developed vitamin D deficiency together with a Fanconi-like syndrome. Four months after having been given oral calcium, phosphate, 1- α -vitamin D and a solution of sodium citrate and citric acid in adequate doses to achieve normal levels of calcium, phosphorus and alkaline phosphatase, she showed no clinical improvement and in addition suffered a crack fracture of the lower left femur. When last assessed her creatinine level was steadily rising. The remaining child, an Indian boy with FGS, had a severe relapse and died in renal failure 10 months after cessation of CyA therapy; while on CyA he did not improve, and his clinical condition fluctuated as it had before treatment with the drug was started. His creatinine value rose gradually, even after cessation of therapy.

Of the remaining 3 children who did not respond to CyA, 1 had a rising creatinine level (above 80 mmol/l) when last examined, and 2 have not attended for follow-up.

CyA dosage and blood levels

Adequate drug levels (trough 250 - 1 000 ng/ml) were achieved in 8 patients at a dosage of 10 - 15 mg/d and in 2 at 7,5 mg/d by 4 - 12 weeks. The patient who was withdrawn by his parent had not attained adequate levels. The 2 patients with MEM who achieved remission on CyA did so at trough levels of 210 - 250 ng/ml and 170 - 300 ng/ml, respectively. The black child with FGS who died 2,5 years after stopping CyA therapy had trough levels of 180 - 280 ng/ml, and peaks of 563 - 1 150 ng/ml. The patient with rickets and chronic renal insufficiency had trough levels of 240 - 280 ng/ml, but peak levels of 1 250 - 1 550 ng/ml. The Indian boy with FGS who died had trough levels of 450 - 875 ng/ml, and peaks of 875 - 1 950 ng/ml.

Toxic effects of cyclosporin during the treatment period

Except for hypertrichosis in 1 patient, other features such as gum hypertrophy, hirsutism and neurological side-effects (tremors, paraesthesiae and convulsions) were not seen in any of the patients. Hepatic dysfunction (according to the definition of a bilirubin level exceeding 17 mmol/l or a 3-fold increase in AST) did not occur, but the serum bilirubin value had increased 2 - 3-fold from baseline levels in 5 of the 9 patients. Similarly, creatinine levels rose 2 - 4 times above baseline values in 5 patients, but did not exceed 160 µmol/l. For the occurrence of nephrotoxicity see below.

Renal biopsies

Five repeat biopsies were available. Three patients had repeat renal biopsies at the end of their 24 weeks of therapy; none showed any histological evidence of acute CyA toxicity (these have been reported on previously⁹). The patient with chronic renal failure and rickets underwent a repeat biopsy 13 months after cessation of CyA therapy. Eleven glomeruli were present. The interstitium showed severe fibrosis with associated tubular atrophy. The fibrosis alternated with bands, giving a striped appearance. The glomeruli showed an increase in matrix and 2 were totally sclerosed; this was compatible with focal segmental glomerular sclerosis. No vascular or tubular epithelial lesions were present. The interstitial changes with associated tubular changes were compatible with chronic cyclosporin toxicity. A postmortem biopsy specimen from the black child who died 2.5 years after cessation of CyA therapy showed end-stage renal damage. Forty-two glomeruli were present; 30 were sclerosed, while the others showed varying degrees of mesangial matrix and cellular increase. Two glomeruli showed pseudotubular formation. These findings supported the diagnosis of focal segmental glomerular sclerosis. The basement membrane was normal and the tubules showed acute tubular necrosis. The interstitium showed a severe diffuse chronic interstitial nephritis with fibrosis, collections of foam cells and tubular necrosis. The vessels were markedly involved, showing hyaline thickening of the walls with narrowed lumina. These are features of focal glomerular sclerosis, end-stage kidney disease with superimposed acute tubular necrosis, and probable cyclosporin toxicity. No renal biopsy specimen was obtained from the Indian boy who died 10 months after cessation of CyA therapy.

Changes in lymphocyte subpopulations over 24 weeks of treatment

Lymphocyte subpopulations were also studied in this group of patients and the findings have been published previously.⁷ There was a tendency for the numbers of T3, T4 and T8 cells and the T4/T8 ratio to regress towards the mean of controls.

Discussion

This pilot study of CyA therapy in 7 black children and 1 Indian child with NS has not provided evidence of any substantial benefit from this drug, which was associated

with serious side-effects in at least 1 of the 8 patients. Difficulties with this study are that the duration of treatment was too long and the trough levels of CyA were too high and potentially toxic in the light of current experience.¹⁰ Further, the incorporation into the study of three different histological types of NS introduced another variable. Paucity of information in 1984 regarding 'safe' trough levels in children influenced therapy and the very serious constraints of conducting such an assessment in a Third-World situation narrowed the choice of suitable participants. It is now known that the adverse renal side-effects of CyA involve both function and structure of the kidney.¹¹

At the completion of a minimum of 24 weeks of CyA treatment in the 8 children available for evaluation, there was no significant change in the features of the NS in 6 patients, and 2 had undergone remission; these 2 children had HBs-positive membranous nephropathy and in 1 the serum went on to clear of HBs and HBe. Improvements in the clinical condition of these 2 patients began before what were at that time regarded as adequate whole-blood trough levels of CyA had been achieved; furthermore, in our experience a third of such patients achieve spontaneous remission, with time, in the absence of specific therapy. Accordingly, it is uncertain whether remission in these 2 children was CyA-induced or part of the natural history of the disease. In neither of them was any drug toxicity produced.

Three of the 6 children whose clinical status had remained unchanged after the use of CyA progressed to chronic renal failure; 2 of these died, both of whom had FGS, a histological group noted for relentless deterioration. It would appear, therefore, that CyA intervention did not alter the expected course in these 2 patients; renal tissue was unavailable in one, and was suggestive of CyA side-effects in the other. One of these 3 children (the black child with an initial diagnosis of MCD but found to have FGS on subsequent biopsy) had, in addition, developed profound tubular damage resulting in Fanconi-type rickets; repeat renal biopsy 13 months after cessation of therapy revealed features considered by one of two pathologists to be probable chronic side-effects of CyA. Renal tubular damage and dysfunction have been documented with CyA therapy,^{12,9} but to the best of our information tubular rickets has not been reported. Low levels of serum calcium and vitamin D occur with NS,¹² but rarely lead to frank rickets. In NS, partial or complete Fanconi's syndrome does occur, but is extremely rare and suggests FGS.¹³ The 3 remaining children in whom repeat renal biopsies were done at the end of 24 weeks showed no evidence of acute CyA toxicity.

There are 3 studies on the use of CyA in children with NS^{3,4,14} with which it is important to compare the above results. Cyclosporin in a dosage of 6 - 11 mg/kg/d, with plasma trough levels of 50 - 150 ng/ml, has been shown by Niaudet *et al.*⁴ to be effective in steroid-sensitive patients with NS, and it was suggested that CyA was indicated in those who had steroid toxicity. However, most patients appeared to relapse on cessation of CyA; furthermore, transient renal insufficiency was evident in 2 steroid-sensitive and 6 steroid-resistant patients. In 5 of these patients renal insufficiency reversed or improved, but, in 1, end-stage renal failure developed within 14 months of the last dose of CyA. This particular case is comparable to our patient who developed chronic renal failure and rickets.

Tejani *et al.*³ reported success with a relatively short (8-week) course of CyA 7 mg/kg/d in steroid-resistant or dependent children with NS classified histologically as FGS, IgM nephropathy and diffuse mesangial proliferative nephritis. From this evidence a response to CyA might have been expected in our 4 FGS patients; none responded or even improved, 2 subsequently died, and 1 developed chronic renal failure with profound tubular damage. Brodehl *et al.*¹⁴ showed that CyA induced remission in steroid-dependent MCD, but relapses recurred on withdrawal of the CyA, and 50% of patients with steroid-resistant FGS responded to CyA.

Sequential studies of patients on CyA have revealed a decrease in the T4/T8 ratio, mainly due to a reduction in T4 cells.¹⁵ However, CyA also inhibits cytotoxic T lymphocytes.¹⁶ CyA appeared to alter numbers of immunoregulatory cells in this study, with changes in the T4/T8 ratios from normal.¹⁷

The tentative implications of this study are that CyA should be used with great caution at lower dosages and lower trough levels for the monitoring of the drug. In FGS, in which prognosis can be poor and newer forms of treatment are needed, these early findings indicate that CyA is not useful and may potentially be harmful.

Relapse of the NS on cessation of CyA therapy is an even greater disadvantage in the South African black child, for whom regular follow-up is a major difficulty. The use of a potentially toxic drug such as CyA for MCD, which has an excellent long-term prognosis, is difficult to justify.

From current knowledge it is now clear that steroid-resistant children with NS are more likely to develop CyA-induced nephrotoxicity,¹⁸ and that the duration of therapy in this study was far too long,¹⁰ thereby increasing this risk.

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The risk of schistosomiasis in Zimbabwean triathletes

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A study was carried out to determine the risk of schistosomiasis in triathletes in Zimbabwe. The prevalence of schistosomiasis in 30 triathletes (24 males, 6 females) was compared with that in 24 non-triathlete controls after the 1989/90 triathlon season. All the subjects found to be infected were then treated with praziquantel (40 mg/kg). The seasonal incidence of schistosomiasis in triathletes was then determined in a prospective study during the 1990/91 season. Schistosomiasis was diagnosed by urine and stool microscopy for ova, blood eosinophil counts and serological bilharzial fluorescent antibody tests for IgM and IgG antibodies. There was a significantly ($P < 0,05$) higher prevalence of schistosomiasis among the triathletes (80%) than among the controls (38%). The seasonal incidence of schistosomiasis was 64%. Exposure of triathletes to fresh-water dam swimming in Zimbabwe poses a significant risk for the development of schistosomiasis.

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In recent years the sport of triathlon (cycling, swimming and running) in Zimbabwe has been characterised by an increasing number of local and overseas competitors who enter the major standard-distance triathlon events on the national race calendar. In the 1989/90 season (October-April) the 1,5 km swim legs of some of the major triathlon events in Zimbabwe were moved away from chlorinated

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