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CLINICAL EFFICACY OF CYCLOSPORIN A NEORAL IN THE TREATMENT OF PAEDIATRIC LUPUS NEPHRITIS WITH HEAVY PROTEINURIA

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SUMMARY

Cyclosporin A (CsA) was introduced in recent years for the treatment of lupus nephritis in patients with steroid resistance or in those with severe corticosteroid toxicity. Our previous study on paediatric patients showed that Neoral (a new microemulsion formulation) had better bioavailability than CsA capsules. To evaluate the clinical efficacy of Neoral in children with lupus nephritis compared with conventional therapy, we performed an open randomized study on 40 children, ranging from 9 to 14 yr old, with class III–V nephritis and heavy proteinuria. They were randomly assigned to either Neoral (5 mg/kg/day), administered q.12 h, or prednisolone (2 mg/kg/day) plus cyclophosphamide (2 mg/kg/day) for 1 yr. Both groups showed a significant decrease in proteinuria (Neoral: 4.62 ± 1.93 to 0.35 ± 0.29 g/day, P < 0.05; prednisolone plus cyclophosphamide: 4.52 ± 1.86 to 0.62 ± 0.21 g/day, P < 0.01). The CH₅₀ haemolytic assay titre decreased after 1 yr of Neoral treatment (26.5 ± 0.9 to 21.4 ± 2.2 U/ml, P < 0.05). Serum C3 and anti-double-stranded (ds) DNA antibody levels also fell with Neoral (C3: 86.2 ± 6.8 to 76.3 ± 4.5 mg/dl; anti-ds DNA antibodies: 14.1 ± 3.2 to 8.2 ± 1.4 IU/ml, P < 0.05). The Neoral group had a significant increase in growth rate over the prednisolone plus cyclophosphamide group (8.2 ± 1.1 cm/yr vs 2.7 ± 0.6 cm/yr, P < 0.01) with improvement of growth status. During the study period, patients tolerated Neoral well with no significant changes in renal function, liver function or lipid profile. Our study implies that Neoral appears to be effective in suppressing proteinuria. Neoral should be regarded as being adjunctive therapy, perhaps with a steroid-sparing effect, in paediatric lupus nephritis. However, its long-term use awaits further studies.

KEY WORDS: Cyclosporin A, Neoral, Lupus nephritis, Heavy proteinuria.

The prognosis of systemic lupus erythematosus (SLE) with lupus nephritis has improved in recent decades [1]. Before 1970, <40% of patients survived for 5 yr [2], while the 10 yr patient and kidney survival rates are now around 90% in some series [1, 3]. Our group showed that the 5 yr renal and patient survival rates of children with class IV diffuse proliferative glomerulonephritis were 87.7 and 82%, respectively [4]. This favourable outcome might be explained by more accurate diagnosis with renal biopsy and more aggressive treatment with immunosuppressive agents. However, the major advance in the treatment of SLE was obtained at the price of side-effects and toxicity of corticosteroids and cytotoxic drugs, such as myelo-suppression, gastrointestinal bleeding, haemorrhagic cystitis and osteoporosis. In paediatric patients, growth retardation is also a serious complication.

In recent years, cyclosporin A (CsA) has been reported as an effective and safer alternative in the treatment of SLE [5]. The main concern about the use of CsA in lupus nephritis is its nephrotoxicity [6]. The nephrotoxicity is related to the serum trough level or area under the curve (AUC) of CsA [7]. Unfortunately, the absorption of oral CsA varies widely, both within patients and between patients [8]. Oral bioavailability averages ~30% [9], with equal bioavailability for the CsA solution and capsule formulation. The actual amount absorbed by patients can vary from <5% to >90% [9, 10]. CsA Neoral, a new microemulsion formulation of CsA, has a markedly less variable pharmacokinetic profile, enabling systemic exposure to be predicted more precisely [11]. There is an improved dose linearity in CsA exposure (AUC), a more consistent absorption profile, and Neoral is less influenced by concomitant food intake and diurnal rhythm [12]. These properties combine to give less intra- and interpatient variability. Up to now, no study of Neoral had been performed in paediatric lupus nephritis. Therefore, we conducted an open randomized trial to evaluate the clinical efficacy of Neoral in paediatric lupus nephritis with heavy proteinuria.

METHODS

From July 1994 to December 1995, 40 children with heavy proteinuria, aged from 10 to 14 yr old, with class III–V lupus nephritis and normal creatinine clearance (CCr) were selected. They fulfilled four or more of the 1982 Revised American Rheumatism Association criteria for SLE [13]. Lupus nephritis was proven by renal biopsy after parental consent during onset of disease. The kidney biopsy specimens were divided into three parts for light, immunofluorescence and electron microscope studies as described previously [4].

Before entering this study, all the patients had received oral prednisolone 2 mg/kg/day for 4 weeks during the acute exacerbation of lupus activity mani-

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fested as nephrotic syndrome, thrombocytopenia, etc. Unresponsible patients subsequently received pulse therapy with i.v. methylprednisolone 20 mg/kg/day for 3 consecutive days and then oral prednisolone 2 mg/kg/day for 1 week as one course. If the patients were still in high lupus activity after the above treatment, further courses of methylprednisolone pulse therapy were continued until control of lupus activity with immunological improvement (elevation of serum C3, C4 level and CH50 haemolytic titre, decrease in anti-double-stranded (ds) DNA antibody level, etc.). We then tapered the dose of prednisolone between 0.5 and 1 mg/kg/day as maintenance therapy for > 1 yr. All of them had persistent heavy proteinuria with 24 h urine protein of >2 g/day and were unable to decrease the dosage of prednisolone further. With the above regimen, all the patients received oral prednisolone with or without pulse therapy for 1–3 yr. They also had growth retardation below –1.5 standard deviation score (SDS) during prednisolone treatment. Mineral supplements including calcium carbonate were prescribed to each patient. Their serum calcium, phosphorus, alkaline phosphatase and intact parathyroid hormone levels were within normal ranges. Their bone age also corresponded to their chronological age. No patient had evidence of delayed puberty.

All the patients were randomly assigned to either CsA Neoral or prednisolone plus cyclophosphamide therapy. The indication for therapy was contained in RESULTS sealed, completely opaque, envelopes numbered in Forty ... both groups are summarized in Table I. There was no significant difference between the dosage of prednisolone further courses of methylprednisolone pulse therapy for 1–3 yr. They also had growth retardation below –1.5 standard deviation score (SDS) during prednisolone treatment. Mineral supplements including calcium carbonate were prescribed to each patient. Their serum calcium, phosphorus, alkaline phosphatase and intact parathyroid hormone levels were within normal ranges. Their bone age also corresponded to their chronological age. No patient had evidence of delayed puberty.

All the patients were randomly assigned to either CsA Neoral or prednisolone plus cyclophosphamide therapy. The indication for therapy was contained in sealed, completely opaque, envelopes numbered in sequence according to a table of random numbers. The enrolment of new patients ended in March 1995, when the planned number of 20 patients in each group was reached. This was considered sufficient to have a power of 0.80 for demonstrating a 0.05 increase in the cumulative proportion of clinical response in the control group vs 0.40 increase in the Neoral group at month 12, using a two-tailed statistical test performed at the 0.05 significance level. In group 1, the patients were started with CsA Neoral 5 mg/kg/day, administered q.12 h; then the doses were adjusted to maintain the trough levels between 75 and 150 ng/ml, as measured by the specific monoclonal radioimmunoassay (RIA) method (INCASTAR CYCLO-Trac SP-Whole Blood Kit, USA) in whole-blood samples taken 12 h after the last dose. The final dose to maintain the above trough level was 3.5–5 mg/kg/day. In group 2, all the patients received prednisolone 2 mg/kg/day plus cyclophosphamide 2 mg/kg/day as initial treatment. We slowly tapered the dose of both drugs to ~0.5–1 mg/kg/day if patients became free of proteinuria. If patients had leucopenia (WBC < 3000/mm3), cyclophosphamide was stopped and steroids were tapered. Furthermore, if patients had signs of infection, prednisolone was stopped. The above regimen lasted for 1 yr. Anti-hypertensive medications, including calcium channel blockers, were given to any patient who developed hypertension during the study period.

The clinical and serological features were recorded during each visit. Serum anti-ds DNA antibody, CH50 haemolytic assay titre and C3 were measured. CCR and 24 h urine protein, haemogram, uric acid, serum creatinine, serum albumin, plasma cholesterol, glucose, transaminases, alkaline phosphatase and total bilirubin were recorded. Anti-ds DNA RIA Kit (Amersham code IM.77/IM.771, USA) was used to detect serum levels of anti-ds DNA for monitoring lupus activity. The CH50 haemolytic assay was carried out using the standard method [4, 14]. Timed urine collections for the determination of CCR were made for 24 h from 7 a.m. to 7 a.m. the next day. The CCR was corrected to a body surface area of 1.73 m2. Hypertension was defined as both systolic and diastolic blood pressure >95th percentile for the respective age groups. Height was measured with a Harpenden stadiometer, always at the same time of the day, and the measurement was repeated until three consecutive readings agreed within a range of 0.2 cm. Baseline mean height was expressed as standard deviation score (SDS) (= height – expected height at that age/s.d. for expected height at that age) [15]. All the above data were recorded each month.

**Statistical analysis**

The statistical significance of differences between groups was evaluated using Student’s t-test. The level of significance was set at α = 0.05. Values are expressed as the mean ± s.d.

**RESULTS**

Forty children with class III–V lupus nephritis with heavy proteinuria were randomized into two groups. The baseline characteristics of both groups are summarized in Table I. There was no significant difference for any item between the two groups. The CCR and liver function were within the normal range in both groups. All had nephrotic-range proteinuria. None had hypertension. The magnitude of proteinuria decreased significantly after 1 yr of treatment in both groups (Neoral: 4.62 ± 1.93 to 0.35 ± 0.29 g/day, P < 0.05; prednisolone plus cyclophosphamide: 4.54 ± 1.86 to 0.40 ± 0.29 g/day, P < 0.05). The indication for therapy was contained in RESULTS sealed, completely opaque, envelopes numbered in sequence according to a table of random numbers. The enrolment of new patients ended in March 1995, when the planned number of 20 patients in each group was reached. This was considered sufficient to have a power of 0.80 for demonstrating a 0.05 increase in the cumulative proportion of clinical response in the control group vs 0.40 increase in the Neoral group at month 12, using a two-tailed statistical test performed at the 0.05 significance level. In group 1, the patients were started with CsA Neoral 5 mg/kg/day, administered q.12 h; then the doses were adjusted to maintain the trough levels between 75 and 150 ng/ml, as measured by the specific monoclonal radioimmunoassay (RIA) method (INCASTAR CYCLO-Trac SP-Whole Blood Kit, USA) in whole-blood samples taken 12 h after the last dose. The final dose to maintain the above trough level was 3.5–5 mg/kg/day. In group 2, all the patients received prednisolone 2 mg/kg/day plus cyclophosphamide 2 mg/kg/day as initial treatment. We slowly tapered the dose of both drugs to ~0.5–1 mg/kg/day if patients became free of proteinuria. If patients had leucopenia (WBC < 3000/mm3), cyclophosphamide was stopped and steroids were tapered. Furthermore, if patients had signs of infection, prednisolone was stopped. The above regimen lasted for 1 yr. Anti-hypertensive medications, including calcium channel blockers, were given to any patient who developed hypertension during the study period.

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**TABLE I**

Characteristics of patients at the time of renal biopsy

<table>
<thead>
<tr>
<th>Item</th>
<th>Neoral n = 20</th>
<th>Prednisolone + cyclophosphamide n = 20</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pathological class</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Class III: 4</td>
<td></td>
<td>Class III: 5</td>
</tr>
<tr>
<td>Class IV: 12</td>
<td></td>
<td>Class IV: 12</td>
</tr>
<tr>
<td>Class V: 4</td>
<td></td>
<td>Class V: 3</td>
</tr>
<tr>
<td>Mean age (yr)</td>
<td>10.2 ± 3.4</td>
<td>10.4 ± 3.1</td>
</tr>
<tr>
<td>Duration of proteinuria (months)</td>
<td>5.6 ± 1.2</td>
<td>5.3 ± 1.4</td>
</tr>
<tr>
<td>Magnitude of proteinuria (g/24 h urine)</td>
<td>4.62 ± 1.93</td>
<td>4.54 ± 1.86</td>
</tr>
<tr>
<td>Serum Cr (mg/dl)</td>
<td>1.1 ± 0.4</td>
<td>1.2 ± 0.3</td>
</tr>
<tr>
<td>CCR (ml/min/1.73 m2)</td>
<td>132.4 ± 9.4</td>
<td>128.5 ± 6.7</td>
</tr>
<tr>
<td>Serum albumin (g/dl)</td>
<td>2.6 ± 0.2</td>
<td>2.5 ± 0.4</td>
</tr>
<tr>
<td>Hypertension</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

All not significant.
Cr, creatinine; CCR, creatinine clearance.
Data are expressed as the mean ± s.d.
Changes in immunological markers and proteinuria between two groups after 1 yr of treatment

<table>
<thead>
<tr>
<th></th>
<th>Neoral</th>
<th>Prednisolone + cyclophosphamide</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n = 18*</td>
<td>n = 20</td>
</tr>
<tr>
<td>Proteinuria (g/24 h urine)</td>
<td>4.62 ± 1.93</td>
<td>4.54 ± 1.86</td>
</tr>
<tr>
<td>CCr (ml/min/1.73 m²)</td>
<td>122.4 ± 19.4</td>
<td>128.5 ± 6.7</td>
</tr>
<tr>
<td>Anti-ds DNA antibodies (IU/ml)</td>
<td>14.1 ± 3.2</td>
<td>15.2 ± 2.3</td>
</tr>
<tr>
<td>CH₅₀ (U/ml)</td>
<td>26.5 ± 0.9</td>
<td>25.2 ± 0.7</td>
</tr>
<tr>
<td>C3 (mg/dl)</td>
<td>86.2 ± 6.8</td>
<td>84.6 ± 5.2</td>
</tr>
</tbody>
</table>

NS, not significant.
Data are expressed as the mean ± s.d.
Proteinuria improved significantly after 1 yr of treatment in both groups.
Immunological parameters decreased after 1 yr with Neoral.
There was no difference in liver function, cholesterol or triglyceride levels after 1 yr of treatment in both groups.

*Two patients were dropped from the Neoral group due to acute exacerbation which needed methylprednisolone pulse therapy.

0.62 ± 0.21 g/day, P < 0.01). No significant decline in CCr was noted with Neoral. The CH₅₀ haemolytic titre decreased after 1 yr of Neoral treatment (26.5 ± 0.9 U/ml to 21.4 ± 2.2 U/ml, P < 0.05) (CH₅₀ normal range: 27.8 ± 0.3 U/ml). A decrease in C3 level was noted in the Neoral group. Both groups showed a significant decrease in anti-ds DNA antibody titre (Table II). In our study, two patients in the Neoral group were withdrawn from the assignment due to acute exacerbation which needed vigorous therapy.

A significant improvement in growth velocity was noted in the Neoral group. All patients had a growth curve below −1.5 SDS before entry into the study. Substitution of Neoral instead of corticosteroids showed recovery of growth velocity, from 2.7 ± 0.6 to 8.2 ± 1.1 cm/year, and catch-up growth height, from −1.6 ± 0.5 to −0.8 ± 0.2 SDS, in group 1 patients (Table III). However, there was no change in growth pattern in group 2 patients.

The CsA-related side-effects were mild. There were no differences in liver function, cholesterol or triglyceride levels after 1 yr of treatment in both groups. Renal function and CCr showed no significant change after Neoral treatment, and the values between the two groups did not differ at any time. Four Neoral-treated patients complained of gingival hyperplasia. The incidence of hirsutism was greater in the Neoral group. Other side-effects, such as hypertension, diabetes mellitus, paraesthesia and tremor, were not observed during treatment.

**DISCUSSION**

In the present study, we proved that Neoral is as effective as conventional therapy in decreasing proteinuria in paediatric lupus nephritis with heavy proteinuria. Moreover, no decrease in glomerular filtration rate (GFR) was noted with Neoral during the study period. However, we did note some patients who showed a decline of GFR during Neoral treatment. Fortunately, normalization of GFR was noted after tapering the dose of CsA. It is well known that CsA may induce functional change even when the therapeutic dose range is prescribed. We did not perform repeated renal biopsies to evaluate the possible renal toxicity of Neoral. However, others have observed long-term renal lesions on repeated biopsies in children following a long course of CsA therapy, even when the initial biopsies were considered normal [16]. If a more prolonged course is given (> 1 yr), renal biopsy is warranted to detect deleterious nephrotoxicity even when the CCr is normal, especially in lupus nephritis.

The mode of action of CsA in reducing proteinuria is yet to be fully elucidated. It has been postulated that CsA may interfere with lymphokine production [17]. An alternative, non-immunological mechanism is by alteration of intrarenal haemodynamics, because

<table>
<thead>
<tr>
<th></th>
<th>Neoral</th>
<th>Prednisolone + cyclophosphamide</th>
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<tr>
<td></td>
<td>Before</td>
<td>After</td>
</tr>
<tr>
<td>Height velocity (cm/year)</td>
<td>2.7 ± 0.6*</td>
<td>8.2 ± 1.1*†</td>
</tr>
<tr>
<td>Height SDS</td>
<td>−1.6 ± 0.5*</td>
<td>−0.8 ± 0.2†</td>
</tr>
</tbody>
</table>

Height SDS, height standard deviation score.
Data are expressed as the mean ± s.d.
All patients suffered from growth retardation below −1.5 SDS before entry into the study.
Growth velocity increased significantly after 1 yr with Neoral, but remained unchanged in the prednisolone + cyclophosphamide group.

*†P < 0.01.
CsA is recognized to cause intrarenal vasoconstriction, probably of the afferent arterioles, associated with a decrease in GFR [18]. More recent work has suggested that CsA restores charge selectivity to the glomerular basement membrane, thereby reducing the membrane permeability [19].

Our data imply a significant decrease in CH₅₀ haemolytic titre in the Neoral group, which was not observed in the prednisolone plus cyclophosphamide group. Moreover, the serum C₃ level decreased with Neoral treatment. However, the level of anti-ds DNA decreased in both groups. It seems that Neoral alone is not effective in controlling serological activity. These results are similar to those reported by Feutren et al. [20]. In 13 steroid-resistant/dependent patients treated with CsA, their mean proteinuria decreased, and lupus serologies (ANA, anti-ds DNA, complement) were unchanged despite clinical improvement [20]. In Favre’s series [5], using CsA with tapering dose of steroids, the majority of the patients did well with clinical remission and a reduction in proteinuria, and stabilization of GFR. He concluded that CsA had a striking effect on proteinuria accompanying both proliferative and membranous glomerulonephritis. However, Tokuda et al. [21] reported that low-dose CsA (3–5 mg/kg/day) with concomitant use of corticosteroids could reduce disease activity as measured by SLE disease activity index along with a reduction in lupus serologies. Thus, while some patients with severe renal and clinical disease are well controlled on CsA, the potential for major disease activation remains in those with persistent serological activity. From our experience, methylprednisolone pulse therapy, followed by i.v. cyclophosphamide, is needed for the control of disease activity during acute exacerbation. CsA is only used in stable patients with heavy proteinuria.

Both proliferative and membranous lupus nephropathy were included in our study, although the latter was felt to exhibit an indolent and relatively benign course. However, progressive renal failure was noted in those with persistent heavy proteinuria [22]. From our experience, patients with class V lupus nephritis need prolonged time for proteinuria to reach nadir. They seemed to have a better prognosis than those in class IV [4]. However, there have been no prospective controlled trials thus far to indicate the risk–benefit ratio of CsA in this disease. Benefits of short-term therapy have been reported in idiopathic membranous nephropathy [23]. The true response rate, optimal dose and duration of action of the drug require further studies.

In conclusion, Neoral appears to be effective in decreasing proteinuria in paediatric patients with lupus nephritis. However, its effect in controlling disease activity remains controversial. Neoral should be envisaged as being adjunctive therapy with steroid-sparing benefits in proliferative and membranous lupus nephritis. However, widespread and long-term use of this potentially nephrotoxic drug in the management of patients with SLE and various forms of lupus nephritis need further controlled trials.

ACKNOWLEDGEMENT

The authors thank Shiu-Chih Tsay for the measurement of serum CH₅₀ haemolytic titres.

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