Renal Biopsy Findings in Children Receiving Long-Term Treatment with Cyclosporine A Given as a Single Daily Dose

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TANAKA, H., TSUGAWA, K., SUZUKI, K. and ITO, E. Renal Biopsy Findings in Children Receiving Long-term Treatment with Cyclosporine A Given as a Single Daily Dose. Tohoku J. Exp. Med., 2006, 209 (3), 191-196 — Long-term treatment of childhood nephrotic syndrome (NS) and rheumatic diseases with cyclosporine A (CsA) given as a single daily dose may yield better results and allow safer use of the drug than the conventional twice-daily dosing. However, the safety of such long-term treatment from the histological standpoint remains to be established. Posttreatment renal biopsy was conducted in a total of eight children (5 with minimal change NS, 2 with focal segmental glomerulosclerosis and 1 with X-linked immune dysregulation, polyendocrinopathy and enteropathy) receiving CsA as a single daily dose, after a mean treatment duration of 20 months (9-36 months). The initial daily dose of CsA (Neoral) was 2.0 mg/kg, given as a single daily dose before breakfast. The dose was subsequently adjusted to achieve a peak (between 1 and 2 hrs post-dosing, C₁-C₂) blood level of around 800 ng/ml. The mean daily CsA dose, mean C₁-C₂ blood level, and mean trough blood level in the subjects were 1.9 ± 0.6 mg/kg, 803.8 ± 117.2 ng/ml and 36.1 ± 12.7 ng/ml, respectively. The result revealed no evidence of CsA-related nephrotoxicity, including arteriopathy, striped interstitial fibrosis or tubular atrophy, in any of the study participants. Also, no significant changes were observed in the mean estimated glomerular filtration rate as compared to the pretreatment values (127.6 ± 14.9 ml/min/1.73 m² vs 115.6 ± 22.8 ml/min/1.73 m²), and except for mild hypertrichosis, no significant adverse effects of CsA were observed. These findings lend further support to the safety of long-term low-dose CsA treatment (median treatment duration in this study, 20 months), with the drug administered as a single daily dose while maintaining a peak (C₁-C₂) blood level of around 800 ng/ml. ——— cyclosporine A; nephrotic syndrome; posttreatment renal biopsy; single daily dose administration
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Following the widespread clinical use of Neoral®, a new oral microemulsion formulation of cyclosporine A (CsA), in place of Sandimmun®, it has been reported that the clinical efficacy of the drug is most significantly related to the peak blood level of the drug in renal transplant patients (Citterio et al. 2001). In this context, we previously reported on the usefulness of low-dose CsA...
(Neoral®) given as a single daily dose, which yielded higher peak blood levels without associated elevation of the trough blood levels of the drug, in selected patients of nephrotic syndrome (NS) in whom CsA administration by the conventional twice-daily administration protocol was associated with a poor steroid-sparing effect (Tanaka et al. 2004; Kudo et al. 2005). However, the safety of our treatment protocol from the histological standpoint remains to be established.

Since CsA nephrotoxicity remains a major problem in CsA treatment (Habib and Niaudet 1994; Inoue et al. 1999; Iijima et al. 2002), in this study, we investigated the safety, from the histological standpoint, of long-term CsA treatment with the drug administered daily as a single-dose while maintaining the peak (between 1 and 2 hrs post-dosing, C1-C2) blood level at around 800 ng/ml, in children with NS and some rheumatic diseases (Kudo et al. 2005; Nakahata et al. 2005; Tanaka et al. 2005).

**PATIENTS AND METHODS**

*Patients*

The definition and criteria used for the diagnosis of steroid-dependent NS (SDNS) and steroid-resistant NS (SRNS), as also for remission and relapse of these conditions, were the same as those described by the International Study of Kidney Disease in Children (Hino et al. 1998; Kano et al. 1999).

From January 2002 to December 2004, 16 children with SDNS, SRNS and X-linked immune dysregulation, polyendocrinopathy and enteropathy (IPEX) were treated at the Department of Pediatrics, Hirosaki University Hospital, with CsA (Neoral) given as a single daily dose while maintaining the peak (between 1 and 2 hours post-dosing, C1-C2) blood level at around 800 ng/ml (Tanaka et al. 2004, 2005; Kudo et al. 2005; Nakahata et al. 2005). Of these, the records of all the 7 children with NS (5 with SDNS and 2 with SRNS) and the child with IPEX, in whom posttreatment renal biopsy could be conducted after long-term treatment with CsA according to our treatment protocol (mean, 20 months; range, 9-36 months) were retrospectively evaluated.

*Treatment protocol*

After obtaining informed consent from the patients’ parents and the approval of the ethics committee at our institution, CsA (Neoral®, Novartis Pharm, Tokyo) therapy was initiated at a daily dose of 2.0 mg/kg, administered as a single dose before breakfast. Measurement of the blood CsA levels was performed every 4 weeks during the study period. The dose was subsequently adjusted to achieve a C1-C2 blood level of approximately 600-800 ng/ml in the SDNS patients (Tanaka et al. 2004; Nakahata et al. 2005), and around 800 ng/ml in the SRNS patients and the IPEX patient (Kudo et al. 2005; Tanaka et al. 2005). We have confirmed in a previous study that most NS children given Neoral® before breakfast show a stable absorption profile of the drug, and also reported that the peak blood levels of the drug are usually achieved between 1 and 2 hrs post-dosing. Thus, we speculated that the CsA blood level measured between 1 and 2 hrs post-dosing (peak blood level) in pediatric patients might show a reliable correlation with the treatment efficacy and toxicity (Nakahata et al. 2005; Tanaka et al. 2005), as reported for the C2 (2 hrs post-dosing) blood level in adult patients (Citterio et al. 2001). Indeed, we have confirmed that in most SDNS patients who receive CsA according to our current treatment protocol a calculated 0-4 hrs area-under-time-concentration curve (AUC0-4) of CsA of around 2,000 ng · hr/ml (data not shown), an adequate value according to a previous report (Uchida et al. 2004), is achieved. However, the most appropriate target AUC0-4 value of CsA for the treatment of relapsing MCNS remains speculative.

For the SDNS patients, CsA administration was started after complete remission had been achieved with prednisolone (PDN); the dose of the concomitantly administered PDN was subsequently tapered according to our treatment protocol for SDNS (Nakahata et al. 2005). For the SRNS patients and the IPEX patient, CsA was administered in combination with PDN at the dose of 0.5-1.0 mg/kg/day. The dose of PDN was subsequently tapered or kept unchanged (Kudo et al. 2005; Tanaka et al. 2005).

*Renal biopsies*

Of the 8 patients included in this study, 7 patients, all except the IPEX patient, had undergone a pretreatment renal biopsy within 6 months prior to the initiation of CsA. The renal biopsy specimens had been examined by light microscopy, immunofluorescence staining, and electron microscopy. All of the 5 patients with SDNS showed histopathological evidence of minimal change disease (MCD), while the 2 patients with SRNS showed lesions characteristic of focal segmental glomeruloscle-
rosis (FSGS). All the 8 patients underwent posttreatment renal biopsy following a mean CsA treatment duration of 20 months (9-36 months), with CsA administered as a single daily dose, in order to investigate the safety of the treatment protocol from the histological standpoint. Tubular atrophy associated with striped interstitial fibrosis is considered to be the most reliable indicator of CsA nephrotoxicity (Habib and Niaudet 1994; Inoue et al. 1999; Kano et al. 1999; Iijima et al. 2002). The severity of the lesion was classified as group I (within normal limits), group II (mild; percentage of the interstitium involved, 1-50%), or group III (moderate or severe; percentage of the interstitium involved, > 50%), according to the modified method described by Habib and Niaudet (1994).

Statistical analysis

Data were expressed as the mean ± s.d., and the statistical analysis was performed by Wilcoxon’s U-test using Stat View Graphics (Abacus Concepts, Berkeley, CA, USA) computer software. A p value of less than 0.05 was considered to denote statistical significance.

RESULTS

The clinical characteristics of the eight study subjects are shown in Table 1. The subjects consisted of six boys and two girls, with a median age at onset of their respective diseases of 5.9 years (range, 2 months-15 years). The median age at the time of initiation of CsA treatment by the single-dose daily protocol was 7.8 years (range, 4 months-15.2 years). All the patients had normal blood pressure and normal values of glomerular filtration rate (GFR) as estimated using Schwartz’s formula (Schwartz 1992), at the time of initiation of the CsA therapy.

The mean CsA dose, C1-C2 blood level and trough blood level in the study participants were 1.9 ± 0.6 mg/kg, 803.8 ± 117.2 ng/ml and 36.1 ± 12.7 ng/ml, respectively (Table 2). The efficacy of CsA treatment using the single daily dose protocol in the 5 SDNS patients, 2 FSGS patients and the IPEX patient has been reported previously (Tsugawa et al. 2004; Kudo et al. 2005; Nakahata et al. 2005; Tanaka et al. 2005). Briefly, CsA was administered as a single daily dose for 9 to 36 months (median, 20 months) in the SDNS patients. Among these, 3 patients (Patients 3, 4 and 5) showed no evidence of relapse even as the PDN dose was tapered. The remaining 2 patients (Patients 1 and 2) showed relapses during the CsA treatment, albeit at a relatively reduced frequency (Nakahata et al. 2005). The minimum dose of PDN required for maintenance of clinical remission and the calculated relapse rate pre- and post-CsA treatment using the current treatment protocol in the 5 SDNS patients are shown in Table 3.

Of the 2 SRNS patients, one (Patient 7) showed a decrease in the urinary protein excretion

<p>| Table 1. Clinical characteristics of 8 patients who received long-term treatment with CsA administered as a single daily dose. |
|---------------------------------|---------------------------------|---------------------------------|---------------------------------|</p>
<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Clinical diagnosis</th>
<th>Age at onset (years)</th>
<th>Age at single-dose daily administration of CsA (years)</th>
<th>Treatment duration (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>SDNS</td>
<td>7</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>2</td>
<td>M</td>
<td>SDNS</td>
<td>15</td>
<td>15.2</td>
<td>15</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>SDNS</td>
<td>2.8</td>
<td>3.7</td>
<td>21</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>SDNS</td>
<td>3.9</td>
<td>8</td>
<td>36</td>
</tr>
<tr>
<td>5</td>
<td>F</td>
<td>SDNS</td>
<td>5.8</td>
<td>6.7</td>
<td>19</td>
</tr>
<tr>
<td>6</td>
<td>M</td>
<td>IPEX</td>
<td>0.2</td>
<td>0.4</td>
<td>33</td>
</tr>
<tr>
<td>7</td>
<td>F</td>
<td>SRNS</td>
<td>3.5</td>
<td>6</td>
<td>13</td>
</tr>
<tr>
<td>8</td>
<td>M</td>
<td>SRNS</td>
<td>9</td>
<td>10</td>
<td>13</td>
</tr>
</tbody>
</table>

F, female; M, male; CsA, cyclosporine A; SDNS, steroid-dependent nephrotic syndrome; SRNS, steroid-resistant nephrotic syndrome; IPEX, X-linked immune dysregulation, polyendocrinopathy and enteropathy.
following CsA treatment using the single daily dose protocol, while the PDN dose was kept unchanged (Kudo et al. 2005); the other patient (Patient 8) showed persistent NS despite 13 months’ treatment with CsA by the single daily dose protocol. This patient was thereafter successfully treated with tacrolimus (Tsugawa et al. 2004). In the IPEX patient (Patient 6), a favorable outcome was achieved with this CsA treatment protocol (Tanaka et al. 2005).

Posttreatment renal biopsy was performed in all the study patients at 9 to 36 months (mean, 20 months) after the initiation of CsA treatment by the single daily dose protocol. Tubular atrophy associated with striped interstitial fibrosis, a typical feature CsA nephrotoxicity (Inoue et al. 1999; Iijima et al. 2002; Nakahata et al. 2005), was not found in any of the study subjects, except one patient with SRNS. The patient with FSGS (Patient 8) showed interstitial lesions, however,
there were mild and graded as group II, and the overall picture suggested a tendency towards improvement as compared to the pretreatment renal biopsy findings (Table 2). Although an increase in the percentage of globally sclerosed glomeruli was observed in one patient with SDNS (Patient 4), neither tubular atrophy nor striped interstitial fibrosis was found in this patient. None of the patients showed any definite hyperplasia of the juxtaglomerular apparatus or arteriolar hyalinosis. At the time of the posttreatment renal biopsy, no significant changes were observed in the mean estimated GFR values as compared with the pretreatment values (127.6 ± 14.9 ml/min/1.73 m² vs 115.6 ± 22.8 ml/min/1.73 m²) in any of the study participants.

**DISCUSSION**

Because of the absence of a reliable correlation between the trough blood level of CsA (Neoral®) and the clinical events in renal transplant patients, it has been suggested that measurement of C₂ (level 2 hrs after the drug administration) might be a simple and useful method for the pharmacokinetic monitoring of CsA; this is based on the observation of a good correlation between the C₂ and the AUC₀⁻¹² (Citterio et al. 2001). We previously reported on the potential usefulness of our current single daily dose protocol for CsA (Neoral®) treatment. Our current treatment protocol yielded higher peak blood levels without associated elevation of the trough blood levels, in selected patients of NS in whom the conventional twice-daily dosing protocol was associated with a poor steroid-sparing effect (Tanaka et al. 2004).

It has been shown that long-term moderate-to low-dose twice-daily CsA dosing protocol to maintain trough blood levels of the drug at around 100 ng/ml (the conventional protocol) is effective and relatively safe for the management of children with NS (Hino et al. 1998; Kano et al. 1999). However, CsA nephrotoxicity remains a major problem (Habib and Niaudet 1994; Inoue et al. 1999; Iijima et al. 2002). In this conventional CsA treatment protocol for NS, Inoue et al. (1999) reported that a young age at the start of CsA treatment and a higher frequency of relapses during CsA treatment might be risk factors for the development of chronic CsA nephrotoxicity. Iijima et al. (2002) recently reported that the duration of CsA treatment and the duration of heavy proteinuria during CsA treatment were significant risk factors for the development of CsA nephrotoxicity. Thus, an optimal CsA treatment strategy for children with NS, with administration of the lowest possible dose of the drug, would be desirable in order to minimize the treatment toxicity, while maintaining the treatment efficacy (Chishti et al. 2001; Nakahata et al. 2005).

Based on the results of our recent preliminary studies (Tanaka et al. 2004; Nakahata et al. 2005), we confirmed that low-dose CsA given as a single daily dose with monitoring of the C₁-C₂ blood level of the drug, is at least as effective, in terms of the steroid-sparing effect, reduction in the frequency of relapses, and no associated increase of the clinical toxicity, as the conventional CsA treatment protocol for children with NS; it is, therefore, a more cost-beneficial protocol than the conventional protocol (Nakahata et al. 2005). Also, this protocol may improve the treatment compliance. However, the safety of this treatment protocol from the histological standpoint remains to be established.

Since CsA-induced tubulointerstitial (TI) lesions characterized by tubular atrophy associated with striped interstitial fibrosis, are considered to represent a typical and irreversible feature of CsA-related nephrotoxicity (Iijima et al. 2002), we paid special attention to the development of these TI lesions in the treated patients. No definite CsA-related nephrotoxicity was observed in any of the study patients. Among the study patients, one patient (Patient 4) with SDNS who received CsA therapy for 36 months showed an increase in the percentage of globally sclerosed glomeruli (6%) without TI lesions, which could be attributable to the long standing SDNS itself. Another patient, with SRNS (Patient 8), showed TI lesions in both the pre- and posttreatment renal biopsies, suggesting that they were probably related to the FSGS itself. Thus, no lesions suggestive of CsA-related nephrotoxicity were observed in any of the posttreatment renal biopsy
specimens. Also, as mentioned before, none of the study patients showed any decrease of the estimated values of the GFR.

In this paper, we retrospectively examined the data of all 8 patients receiving long-term treatment with low-dose CsA administered as a single daily dose with the C1-C2 blood level maintained at around 800 ng/ml, in whom posttreatment renal biopsy could be conducted. Despite the limitations of the study, that is, the small number of study subjects, relatively short observation period, and differences in the underlying diseases and timing of the posttreatment renal biopsy, we believe that our study results represent useful and interesting preliminary results that may be validated in future studies.

We, therefore, conclude that these preliminary findings may lend further support to the evidence of the histological safety of long-term CsA treatment (median duration, 20 months) with the drug administered as a single daily dose while maintaining the C1-C2 blood level at around 800 ng/ml. However, further studies in larger numbers of patients are needed to confirm the results.

References


