Treatment of severe steroid-dependent nephrotic syndrome (SDNS) in children with tacrolimus

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Abstract

Background. Severe steroid-dependent nephrotic syndrome (SDNS) is a common type of nephrotic syndrome (NS) observed in childhood. Steroid-sparing agents such as calcineurin inhibitors (CNIs) are used to avoid steroid toxicity in SDNS. Tacrolimus (TAC) has been prescribed for maintaining remission of NS in patients who have developed treatment resistance or adverse effects with cyclosporin A (CYA) at our institution since 1995. The aim of this study was to compare the efficacy and complications of TAC with CYA in the management of severe SDNS.

Methods. We report a retrospective longitudinal clinical series of patients with SDNS, all of whom have been treated with TAC.

Results. Ten SDNS children (eight males) were reviewed quarterly from time of initial referral to the present day during 93 completed treatment patient years. Nine patients had minimal change disease and one had focal segmental glomerulosclerosis on their first biopsy. The median age at diagnosis was 2.9 years (range 1.6–12.9). The median age at initial referral was 3.9 years (range 2.2–12.9). All patients initially responded to prednisolone at 60 mg/m²/day, and subsequent frequent relapses were treated sequentially with oral cyclophosphamide 168 mg/kg over 8–12 weeks (n = 10), CYA (n = 10), intravenous mustine or a second course of cyclophosphamide (n = 7) and then TAC (n = 10). The initial daily treatment of CYA and TAC in two divided doses was 5 and 0.1 mg/kg/day, respectively; targeted 12 h blood drug levels were of 50–100 µg/l for CYA and 5–10 µg/l for TAC. Patients underwent renal biopsy and the formal glomerular filtration rate (GFR) was measured using plasma clearance of the Inutest/C213 method every 2–3 years while receiving CNIs. Six patients continued with TAC; in four patients, TAC was discontinued because of poor response (n = 2), hypertension (n = 1) and glucose intolerance (n = 1). For CYA and TAC treatment periods, the median NS relapse rate was two and one relapses per year, respectively, and cumulative steroid dosage was 73.9 and 105.2 mg/kg/day, respectively (P = 0.54). The reduction in GFR was 5.8 and 11.7 ml/min/1.73 m² during these periods. Three of the 10 patients showed histological evidence of mild CNI nephrotoxicity over the whole of the CNI treatment period despite achieving target therapeutic drug levels; no significant change in measured or calculated GFR over this prolonged CNI therapy was observed. Antihypertensive medication was prescribed for 11 of 31 CYA and 22 of 40 TAC treatment years. Growth was maintained during the entire CNI therapy period with median change in height SD scores (SDS) of +0.37 and −0.03 over CYA and TAC, respectively (P = 0.13).

Conclusion. In conclusion, we observed that the replacement of CYA by TAC does not lead to a better management of severe SDNS.

Keywords: calcineurin inhibitors; children; cyclosporin A; nephrotic syndrome; steroid dependency; tacrolimus

Introduction

Steroid-sensitive nephrotic syndrome (SSNS) remains the predominant type of nephrotic syndrome (NS) involving children, and after 8 weeks of prednisolone therapy >50% of children will relapse and require further courses of steroid therapy [1–3]. Steroid dependency has been defined by the International Study for Kidney Diseases in Children [4] (ISKDC) as children with frequently relapsing nephrotic syndrome (FRNS) in whom two consecutive relapses, or two of four relapses in any 6 month period, occurred while still on a dose of steroids or within 14 days of discontinuing...
steroid therapy. The rate of steroid dependency has been quoted as >30%, but depends on referral patterns to paediatric renal centres. Although treatment with glucocorticoids remains the mainstay of therapy for these patients, significant morbidity is associated with prolonged glucocorticoid therapy. ‘Steroid-sparing’ agents such as alkylating agents initially and then, if further frequent relapses recur, calcineurin inhibitors (CNIs) are used to reduce glucocorticoid dosage and consequently their side effects. The use of cyclosporin A (CYA) in SDNS has been well described previously [5–7]. Evidence for the use of tacrolimus (TAC) for SDNS in the literature is sparse, being confined to small series or single case reports with the biggest series involving adult patients and a recent report of early experience with TAC in paediatric patients with SDNS and focal segmental glomerulosclerosis (FSGS) [8–13]. These reports showed a superior anti-proteinuric effect of TAC when compared with CYA, with better short-term control of SDNS patients, predominantly with FSGS.

TAC has been used in our unit for SDNS since 1995. We report here our experience in the management of severe SDNS with TAC. The aim of our study was to compare the efficacy and complications of TAC with those of CYA in SDNS.

Patients and methods

A retrospective longitudinal case study was performed on 10 patients who presented with SDNS to the paediatric renal unit at Guys Hospital, London. Data from case notes at our and other hospitals were analysed from the time of referral to 31 December, 2002. The ISKDC definition of SDNS was confirmed in all patients. All patients with SSNS with a subsequent steroid-dependent clinical course requiring TAC therapy were included. Patients with steroid-resistant nephrotic syndrome were excluded.

Clinical management

All patients after presentation were seen at least quarterly. At each clinic visit, patients had their height and weight recorded. Clinic review included recording of any relapses of NS, steroid regimen used, days to achieve remission and weaning regimen of steroid therapy. Blood pressure was recorded using an appropriately sized cuff and a mercury sphygmomanometer, and assessed using updated 2nd Task force blood pressure criteria [14]. Qualitative urinalysis was performed for evidence of proteinuria (recorded as negative, trace, 1+, 2+, or 3+) and measurements of plasma creatinine, plasma albumin and trough blood CYA or TAC levels were carried out.

After referral, all patients were sequentially treated initially with oral daily cyclophosphamide at a total dose of 168 mg/kg over 8–12 weeks. Repeat relapses thereafter were managed with CYA 5 mg/kg/day in two divided doses. Relapses while on CYA were managed with oral prednisolone (60 mg/m²/day) and, if control of the nephrotic relapses was thought to be inadequate, a second course of alkylating agent therapy was used (oral cyclophosphamide, n = 2; or intravenous mustine, n = 5). Subsequent failure to maintain the patient in remission was then managed by prescribing TAC 0.1 mg/kg/day in two divided doses. Further changes to CNI dose were made on the basis of 12h trough blood levels of 50–100 µg/l for CYA and 5–10 µg/l for TAC. Drug levels were measured at least 4 monthly. The CYA assay involved extraction of CYA from red blood cells as blood proteins are precipitated during the preparation steps. The supernatant is then analysed on a Cobas Mira analyser using the Behring Cyclosporin 2000 EMIT assay. TAC was analysed on the Abbott IMX analyser. The IMX Tacrolimus II assay is based on microparticle enzyme immunoassay technology. Prior to analysis on the IMX system, a manual pre-treatment step is performed in which the whole blood sample is extracted with a precipitating reagent and centrifuged. The supernatant is decanted into a sample well and analysed on the IMX system.

In the period between the first course of cyclophosphamide and commencing CYA and starting TAC, patients received various therapies—levamisole (n = 8; 9 treatment years), mycophenolate mofetil (MMF, n = 4; 2.4 treatment years), azathioprine (n = 2; 1.2 treatment years) and a combined course of vincristine, cyclophosphamide and prednisolone (n = 5).

All patients underwent renal biopsies every 2–3 years and also measurement of their glomerular filtration rate (GFR) using the plasma clearance of the InuTest® method. Renal biopsies were performed to detect histological evidence of CNI toxicity. At the time of renal biopsy and GFR measurement, none of the patients were in clinical or biochemical relapse. GFR was also calculated by the method of Schwartz & al. [15].

Indications for TAC therapy

TAC was prescribed for those patients if further use of CYA was not possible because there was lack of continuing response to CYA therapy and/or the patients developed adverse effects of CYA. A lack of response was noted if patients had failed to maintain a sustained remission after 1 year on CYA or if, while taking CYA therapy, they had frequent relapses requiring escalating doses of glucocorticoids to maintain remission. CYA was also stopped if adverse effects such as severe cushingoid appearances, hypertension and deterioration in GFR or histological evidence of CNI-induced nephrotoxicity were observed.

Data analysis

The clinical data obtained are analysed as comparisons of the two therapy periods on CYA and TAC with respect to efficacy and complications of drug therapy. Efficacy of the two treatment periods on CYA and TAC was assessed in terms of: (i) number of relapses per year; and (ii) amount of glucocorticoid prescribed in mg/kg/year. Complications were analysed in terms of: (i) the effect of drug therapy on renal function as measured by both plasma clearance of the InuTest® injection method and calculated GFR; (ii) histological evidence of CNI nephrotoxicity; (iii) effect of drug therapy on blood pressure; and (iv) statural growth. Any significant side effects while on drug therapy were recorded.

Mean, median, SD and SEM were calculated for treatment periods on CYA and TAC and were compared for differences...
using the two-tailed paired t-test method and the Wilcoxon signed ranks test (SPSS v9 statistics program).

**Results**

Ten patients (eight male) were followed for 93 completed patient treatment years; nine patients had minimal change disease (MCD) at the time of presentation and one patient had FSGS.

The median age (range) of the patients at initial referral was 3.9 years (2.2–12.9). The median age at start of CYA treatment was 4.9 years (2.6–13.4) and that for TAC 10.9 years (3.6–21.4) \((P<0.01)\). The median interval after referral to initiation of CYA therapy was 0.8 years (0.1–6.4) and median interval between stopping of CYA and beginning TAC was 0.6 years (0.01–2.4). There were 16 years in the pre-CYA period and 6 years in the post-CYA but pre TAC-period.

The median duration of CYA therapy was 2 years (1–7) and subsequently of TAC 5 years (1–7) \((P=0.49)\). This gave cumulative patient years on CYA of 31 years and that on TAC of 40 years. The median duration on CNI medication (combined CYA and TAC) was 7.5 years (2–13).

TAC therapy was indicated if there was lack of response to CYA \((n=8)\) or if there were adverse effects while taking CYA \((n=6)\). After a period of 1 year on CYA, a lack of response was considered if patients had failed to maintain a sustained remission \((n=4)\) or if they had frequent relapses requiring increasing doses of glucocorticoids to maintain remission \((n=4)\). Six patients had adverse effects while on CYA therapy; deterioration in calculated GFR \((n=4)\), histological evidence of CNI toxicity \((n=1)\) and new onset of hypertension \((n=1)\). Four of the 10 patients had both a lack of response and adverse effects while on CYA therapy; three had reversible reductions of GFR and one had onset of new hypertension. The calculated GFR improved in all three patients once CYA was stopped. The patient who became hypertensive on CYA had normal blood pressure while taking TAC and did not require any antihypertensive medication subsequently.

Overall, six patients continued and four patients discontinued TAC therapy. In two patients there was poor disease control, one patient developed hypertension after 1 year of TAC therapy but became normotensive once TAC was discontinued and another patient developed insulin-dependent diabetes mellitus (IDDM) after 4.3 years on TAC therapy. This patient remains insulin dependent. The two patients with a poor response to TAC were subsequently treated with MMF; one of these patients went on to have bilateral nephrectomy and a renal transplant and the other remains stable on a second course of CYA treatment.

**Efficacy of CNI treatment**

There were no observed differences in the efficacy of CNI therapy for the two treatment periods (Table 1). The annual relapse rate for these patients with severe SDNS was low while taking CNI treatment and not statistically different on comparison of the two drug therapy periods \((P=0.79)\). Whether treated with CYA or TAC, the amount of glucocorticoid prescribed was modest and did not differ significantly between the two therapy periods on CYA and TAC \((73.9 \ vs \ 105.2 \ mg/kg/year; \ P=0.54)\). No reduction in maintenance dose of glucocorticoid occurred with TAC.

**Complications of CNI therapy**

Table 1 also shows data comparing complications while on treatment with CYA or TAC. In addition to 4 monthly plasma creatinine measurements, all our patients had measurements of GFR by the plasma clearance of Inutest® method along with a percutaneous renal biopsy every 2–3 years.

The effect of CNI therapy on renal function was assessed by analysing changes in calculated GFR using the Schwartz formula just prior to start of CNI therapy, at 3 months on therapy and at the end of CNI therapy. Before beginning CNI therapy, the median calculated GFR in ml/min/1.73 m² was 105.7 for CYA and 101.8 for TAC. Three months after starting CYA and TAC, the percentage calculated GFR fell by median of −5.3 and −4.0%, respectively, which is not statistically significantly different \((P=0.91)\). Over the duration of CYA and TAC treatments, the percentage calculated GFR fell by −5.8 and −11.7%, respectively, comparison of this effect for the two drugs also did not

**Table 1. Data comparing the efficacy and complications during the two therapy periods on CYA and TAC**

<table>
<thead>
<tr>
<th></th>
<th>CYA ((n=10))</th>
<th>TAC ((n=10))</th>
<th>(P)-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Rate of relapse/year, median (range)</td>
<td>3 (0–6)</td>
<td>1 (0–5)</td>
<td>0.79</td>
</tr>
<tr>
<td>Glucocorticoid in mg/kg/year, median (range)</td>
<td>73.9 (2.1–468.9)</td>
<td>105.2 (17.3–602.7)</td>
<td>0.54</td>
</tr>
<tr>
<td>Percentage change in calculated GFR in ml/min/1.73 m² (median, range)</td>
<td>−5.8 (−37.7, +38.3)</td>
<td>−11.7 (−34.3, +3.5)</td>
<td>0.46</td>
</tr>
<tr>
<td>Histology, CNI toxicity</td>
<td>3/16 (2 patients)</td>
<td>1/13 (1 patient)</td>
<td>–</td>
</tr>
<tr>
<td>Height SDS pre-therapy (median, range)</td>
<td>−0.68 (−1.67, +1.75)</td>
<td>−0.60 (−1.99, +0.49)</td>
<td>0.61</td>
</tr>
<tr>
<td>Height SDS post-therapy (median, range)</td>
<td>−0.22 (−1.07, +1.33)</td>
<td>−0.70 (−1.88, +1.45)</td>
<td>0.13*</td>
</tr>
<tr>
<td>Treatment years on antihypertensive ((n=7))</td>
<td>11</td>
<td>22</td>
<td>–</td>
</tr>
</tbody>
</table>

*Denotes change in Ht SDS between start and finish for both CYA and TAC.
show a statistically significant difference \((P = 0.46)\). The calculated GFR at the initiation of TAC was comparable with that with CYA despite the fact that patients had received CYA for a median duration of 2 years prior to starting TAC.

**Renal function and blood pressure**

Thirty-five Inutest® GFR measurements were performed on the 10 patients in remission during 71 CNI treatment years. The Inutest® GFR measurements for all 10 patients over the entire CNI therapy period (CYA + TAC) are shown in Figure 1, including those performed when there was evidence of nephrotoxicity on biopsy. Two of 35 measured GFRs were below 80 ml/min/1.73 m², the lower limit of normal, in two patients; there was no histological evidence of nephrotoxicity around the time of these two measurements on biopsy which were performed 8 months previously and 10 months later. Seven calculated GFRs were below the normal limit in four patients; measured GFR was low on only one measurement at this time. There was a lack of correlation between calculated GFR and the Inutest® GFR measurements \((R^2 = 0.035)\). Furthermore, five of the Inutest® GFR determinations revealed hyperfiltration (defined here as >140 ml/min/1.73 m²) and six measured GFRs had values between 120 and 140 ml/min/1.73 m². No hyperfiltration was observed by calculated GFR methods.

In a further analysis of measured GFR for the whole period spent on CNI therapy, the period has been arbitrarily divided into 2 year intervals. No significant decline in GFR was demonstrated over 10 combined years of CNI treatment in these SDNS children.

Seven of the 10 patients required antihypertensives although none of the patients were on any antihypertensive medication before starting CNI therapy. The same seven patients required antihypertensive medications on both CYA and TAC. Two further patients developed new onset hypertension as a result of CNI therapy. These two patients became normotensive and have remained so once CNI therapy was stopped.

**Renal histology**

Twenty-nine biopsies were performed in these 10 patients while taking CNI therapy. Evidence of CNI toxicity was observed on four biopsies in three patients; three biopsies affecting two patients while on CYA and one biopsy affecting one patient during TAC treatment, not previously known to have CNI nephrotoxicity. One patient had two biopsies with CNI toxicity after 1.3 and 4.8 years on CYA; the other patient had CNI toxicity after 4.3 years on CYA. Both these patients had mild histological evidence of CNI toxicity with peritubular capillary thrombi with a well preserved interstitium. Neither had a change in calculated GFR. They both underwent subsequent

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**Fig. 1.** Box plot of formal GFR measurements. The line through the box represents the median, and the whiskers represent the upper and lower ranges of the data set during the given 2 year treatment period. ‘n’ represents number of Inutest® measurements.
biopsies that have shown no permanent damage due to CNI treatment.

The patient with evidence of CNI toxicity while taking TAC for 5 years had intimal fibrosis involving small and medium arteries and tubular vacuolation. This patient had previously taken CYA for 4 years with no histological evidence of CNI toxicity and has not as yet had another biopsy. The calculated GFR and measured Inutest® GFR were maintained. The CNI drug levels were within the targeted therapeutic range around the time of the biopsies.

Growth

Growth was maintained during the entire therapy period on CNIs and there was no significant difference in the height SD scores (SDS) at the start of CYA therapy and at the end of TAC therapy ($P = 0.62$). Growth was maintained during the entire CNI therapy period with median change in height SDS of $+0.37$ and $-0.03$ over CYA and TAC, respectively ($P = 0.13$). During treatment on CYA, there was an improvement in median height SDS from $-0.68$ to $-0.22$ ($P = 0.05$) but this gain had been lost by the time TAC therapy was initiated (median height SDS $-0.60$; $P = 0.01$); while on TAC, the median height SDS failed to show any improvement ($-0.60$ to $-0.70$; $P = 0.98$).

Discussion

We have reported a retrospective longitudinal clinical series of 10 children with severe SDNS who were treated with both CYA and TAC sequentially and were closely monitored for 91 patient years. Although this study was non-randomized with respect to which CNI was prescribed first, our results demonstrate no significant difference in efficacy, cumulative steroid dosage, renal function or statural growth between CYA and TAC therapies in SDNS. Renal function measured by plasma Inutest® clearance is preserved over a 10 year period of combined CNI treatment in this disease. The low prevalence of CNI toxicity observed on renal histology supports our functional data about long-term safety of CNIs in SDNS.

Children with severe SDNS and MCD form a small but important group of patients with idiopathic NS. Their clinical management poses considerable problems—that of disease control, effects on growth and adverse effects of drugs, such as changes in appearance, renal function and blood pressure. In general, our patients were managed in a manner similar to that reported by Neuhaus et al. [16] in order to maintain their NS in remission and minimize drug side effects. In such severe SDNS with MCD patients (only one of our patients had steroid-sensitive FSGS), CNIs have been the mainstay of therapy for many years [5]. With the introduction of TAC, we hoped to produce lower annualized relapse rates, a reduced requirement for steroids while maintaining normal renal function and minimal nephrotoxicity, and improving the cosmetic side effects of CYA.

The basis of TAC’s action in SDNS is unknown, but its immunosuppressive effects are regarded as most important. The early use of TAC to control SDNS reduced relapses [10,12]. TAC achieved a superior anti-proteinuria profile but by a biological mechanism that remains unknown; investigators have associated this superior control of proteinuria with TAC’s more potent effect on the release of cytokines such as interleukin-8 (IL-8) [17], on its suppression of vascular permeability factor (VPF) production [18] or its action on intraglomerular haemodynamics [19]. We confirmed good
control of SDNS with the low annualized relapse rate observed in our study.

Our analysis of the annual relapse rate demonstrated no advantage of TAC over CYA, unlike the report of Duncan et al. [13], and no correlation between the length of time on CNI therapy and relapse rate. This difference may be explained by the fact that their patients were adult nephrotic patients with FSGS. We observed no benefit in reduced glucocorticoid medication with TAC treatment either. Individual patients showed a varied response from one year to the next despite maintaining targeted blood drug levels. CNI agents were able to control disease better when compared with other steroid-sparing agents that were used immediately before the initiation of CYA or TAC (data not shown).

Complications such as nephrotoxicity and hypertension from CNI medication raise important clinical considerations, as SDNS in children is not usually associated with permanent renal impairment or hypertension. Our results on GFR showed that firstly there was poor correlation of measured Inutest® GFR with calculated GFR. Abnormally low calculated GFR results occurred when measured GFR values were within the normal range, making reliance on plasma creatinine values for monitoring purposes doubtful in SDNS patients treated with long-term CNIs. This is demonstrated in Figures 1 and 2. In our case series, patients who stopped CYA because of concerns of nephrotoxicity did not have a true GFR measured at the time, which is a shortcoming of this study. It is important to note that evidence for drug toxicity was seen histologically despite us achieving targeted drug levels and no significant change in calculated or measured GFR over prolonged periods of CNI therapy. When low GFR results were obtained by either method, only two out of seven renal biopsies revealed histological CNI toxicity, suggesting that measurement of Inutest® GFR to demonstrate deteriorating renal function as well as renal biopsy for CNI histological changes to monitor renal injury are required in SDNS treated with CNIs.

All patients in our series maintained growth during the entire therapy period, but there were differences in height SDS while patients were on CYA and TAC. During treatment on CYA, there was an improvement in median height SDS from −0.68 to −0.22 ($P = 0.05$); while on TAC the median height SDS failed to show any improvement (−0.60 to −0.70; $P = 0.98$). This may have been because the patients were older, peripubertal and in boys especially, had continued on steroid therapy.

Reports in the literature have also expressed concerns on the long-term effect of CNI agents on renal function. In nephrotic children, measured GFR was observed to decrease with long-term CYA treatment [5]. The Inutest® GFR measurements shown in Figure 1 demonstrate that, despite prolonged treatment with CNIs, the median GFR remained unchanged, suggesting no evidence of functional nephrotoxicity. Gellerman et al. [20] have reported similar data on measured GFR, which remains stable, but there is a reduction in effective renal plasma flow (ERPF).

Four patients who showed a lack of response to CYA with no sustained remission after 1 year of CYA therapy did go on to respond to TAC and prednisolone subsequently. CYA was stopped because they had frequent relapses requiring increasing doses of glucocorticoids to maintain remission. These four patients had at the time of review been on TAC and prednisolone for a median of 5 years (1–7) and three of these four had associated adverse effects on CYA but not on TAC. The observation in the remaining six patients is consistent with the proposed superior antiproteinuric effect of TAC [10]. The two patients in whom stopping CYA was indicated because of the onset of adverse effects had received CYA for 6 and 7 years. These patients responded poorly to TAC and prednisolone but did not have recurrence of CNI adverse effects. Thus, SDNS patients showed no predictable response to TAC therapy after CYA, but CYA side effects may be minimized with TAC.

CNI agents offer good control of SDNS. In our experience with a small number of children with severe SDNS who failed to show a sustained remission after 1 year of CYA therapy, TAC proved beneficial in some. Patients should be monitored for CNI nephrotoxicity by functional and histological investigations, but it is uncommon even after long-term CNI treatment. No significant advantage of TAC over CYA was demonstrated apart from some improvement in cosmetic appearances. In conclusion, we observed that the replacement of CYA by TAC does not lead to a better management of severe SDNS.

References

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Conflict of interest statement. None declared.

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