Tacrolimus: a new therapy for steroid-resistant nephrotic syndrome in children

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Abstract
This study was conducted to evaluate the safety and efficacy of tacrolimus (TAC) in children with SRNS. The study group comprised of 22 consecutive children with steroid-resistant nephrotic syndrome (SRNS) who were studied prospectively. TAC was initiated with a dose of 0.10 mg/kg/day, and the dose was increased to attain a trough level of 5.0–10.0 g/l. These patients were treated with concomitant prednisone, which was subsequently tapered off and stopped. The primary outcome variable was the number of patients who attained a complete remission (CR) or partial remission (PR). The mean age of onset was 7.33 ± 5.9 years, and there were 20 boys and 2 girls. Of the 22 children, 9 had minimal change disease, 11 had focal segmental glomerulosclerosis and the other 2 had diffuse mesangial hypercellularity on histopathology. TAC had to be withdrawn in 3 children because of its side effects. Of the remaining 19 children who received adequate therapy and were able to achieve target levels, CR was seen in 16 (84%) children, 2 (10.5%) attained PR and 1 was nonresponsive. The mean time to achieve remission was 63.2 ± 44 days and the mean dose of TAC was 0.18 ± 0.07 mg/kg.

The mean urine spot protein/creatinine ratios were significantly lower (0.33 ± 0.58 vs. 13.5 ± 21.9 mg/mg, p = 0.002) and the mean serum albumin levels were significantly higher (3.92 ± 0.35 g/dl vs. 2.39 ± 0.56 g/dl, p = 0.00005), as compared to those prior to starting TAC. The mean glomerular filtration rate values at the end of the study were similar to those prior to starting TAC (97.9 ± 21.2 ml/min/1.73m² vs. 96.4 ± 18.4 ml/min/1.73m², p = 0.30). The mean duration of follow-up was 290 ± 126 days. This is the largest study so far on the safety and efficacy of TAC therapy in SRNS. Our results suggest that TAC is an effective therapeutic modality for SRNS, including the subgroup of children who are nonresponsive to the current therapeutic modalities like cyclophosphamide and cyclosporine.

Keywords: cyclophosphamide; cyclosporine; focal segmental glomerulosclerosis; minimal change disease; steroid-resistant nephrotic syndrome; tacrolimus

Introduction
The management of steroid-resistant nephrotic syndrome (SRNS) continues to pose a therapeutic challenge [1]. The various therapeutic options include cyclophosphamide, cyclosporine, intravenous methylprednisolone, angiotensin-converting enzyme inhibitors (ACEI) and mycophenolate mofetil [1–7]. Most studies have reported a success of 50–60% [1]. In the case of persistent proteinuria, the prognosis is poor with a high risk of progression to chronic renal failure [1,8,9].

Tacrolimus (TAC) is a calcineurin inhibitor that is more potent in cytokine suppression than cyclosporine [10]. Another advantage of TAC is the lesser nephrotoxic potential as compared to cyclosporine. There have been few reports on the use of TAC in children. Most of these case series were small, heterogeneous and have included children with steroid-dependent nephrotic syndrome and SRNS [11–15]. This study was conducted to evaluate the safety and efficacy of TAC in children with SRNS.

Patients and methods
The study group comprised 22 consecutive children with SRNS who were referred to our institute over the last one year. They were studied prospectively. At presentation, they were evaluated clinically for hypertension, haematuria, anthropometric parameters (height, weight and body surface area) and systemic involvement. They were investigated for confirmation of nephrotic syndrome, renal function status, as well as evaluation of secondary causes of nephrotic syndrome and hepatitis B surface antigen (HBsAg), hepatitis C virus (HCV) or human immunodeficiency virus (HIV) seropositivity. All cases fulfilled the International Study of Kidney Disease in Children criteria for the diagnosis of

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nephrotic syndrome [16]. The study was approved by the Institute Ethics Committee.

After an informed consent, all children were subjected to a renal biopsy via percutaneous route under ultrasound guidance. All kidney samples were sent for routine light microscopy, immunofluorescence and electron microscopy. The biopsy specimens were reviewed and interpreted by the same pathologist, and the histopathological diagnosis was made as per the standard case definitions defined in our earlier study [17]. For the purpose of this study, we included only children who had minimal change disease (MCD), focal segmental glomerulosclerosis (FSGS) and diffuse mesangial hypercellularity (DMH).

TAC was initiated with a dose of 0.10 mg/kg/day divided into two doses over 12 h intervals. The dose was increased to attain a trough TAC level of 5.0–0.0 g/l [10–14]. The duration of the planned therapy was 1 year. Of the 22 children, 14 had previously been treated with other immunosuppressive agents; 5 had been given intravenous cyclophosphamide in a dose of 500 mg/m²/month x 3 doses while another 9 had received oral cyclophosphamide in a dose of 2.5 mg/kg/day for 3 months. Four of these children had received prior cyclosporine therapy in a dose of 5 mg/kg/day for 6 months without response. All previous immunosuppressive agents were discontinued and these children were given a washout period of 3 months prior to the start of TAC. These patients were treated with concomitant prednisone in a dose of 60 mg/m²/day for 4 weeks, followed by 40 mg/m² every alternate day for 4 weeks and tapered over the next 4 weeks. Children who did not respond to 6 months of TAC were labeled as TAC resistant. Patients who relapsed while on TAC were treated with the standard dosing of prednisone (60 mg/m²) until remission, followed by a gradual tapering over 2 months.

These children were followed-up twice weekly initially for the first month, followed by monthly visits. They were subjected to a baseline assessment of standard biochemical parameters: glucose, creatinine, urea, electrolytes, albumin and complete blood count. Urine spot protein creatinine estimation was done initially. Blood was drawn for measurement of TAC trough levels 2 weeks after the initiation of TAC. A repeat level was done in those children who required dose modifications based on the target TAC levels. TAC levels were measured via the IMX analyzer utilizing the micro-particle enzyme assay (MEIA) (Abbott Laboratories, Wiesbaden, Germany). On follow-up, each patient was subjected to clinical examination, including height, weight and blood pressure measurement. A repeat urine spot protein/creatinine ratio (Pr/Cr), blood glucose, serum protein, albumin and creatinine estimation was also done at each follow-up visit.

The primary outcome variable was the number of patients who attained a complete remission (CR) or partial remission (PR). Secondary outcome variables included renal function during treatment, adverse events, TAC dosing and levels, time to achieve remission and maintenance of remission once achieved.

The following case definitions were used for the purpose of this study. Nephrotic syndrome was defined as a syndrome comprising of hypoalbuminemia (<30 g/l), hyperlipidemia (cholesterol > upper limit of normal for age), oedema, and proteinuria (urine Pr/Cr > 2 mg/mg). Steroid resistance was defined as no clinical response after 8 weeks of daily steroids at 60 mg/m² per day (maximum dose 80 mg/day) [16]. A relapse was defined as urine dipstick of >3+ with no previous proteinuria and with clinical evidence of oedema or dipstick of >2+ proteinuria for 3 days. CR was defined as a normal spot urine Pr/Cr (0.2 mg/mg) and/or a negative urine dipstick for protein for 3 days or more. PR was defined as spot urine Pr/Cr ratio between 0.2 and 2 g/mg and absence of oedema. Nephrotic-range proteinuria was defined as urine Pr/Cr ratio > 2 mg/mg [16]. Hypertension was defined as a systolic blood pressure or a diastolic blood pressure greater than the 95th percentile for age and sex measured on at least three separate occasions [18]. Glomerular filtration rate (eGFR) was calculated by the Schwartz formula [19].

**Results**

The mean age of onset of nephrotic syndrome in the study group was 7.33 ± 5.9 years, and there were 20 boys and 2 girls. Of the 22 children, 9 had MCD, 11 had FSGS and the other 2 had DMH on histopathology. Of these, 19 were primary steroid resistant, while 3 had secondary steroid resistance. TAC had to be withdrawn in 3 children: 1 developed haemolytic uremic syndrome (HUS), another developed glucose intolerance, while the third child developed diarrhoea and was unable to attain therapeutic TAC levels. Of the remaining 19 children who received adequate therapy and were able to achieve target levels, CR was seen in 16 (84%) children, 2 (10.5%) attained PR and 1 was non-responsive. The mean time to achieve remission was 63.2 ± 44 days, and the mean dose of TAC was 0.18 ± 0.07 mg/kg. The mean trough levels were 9.54 ± 5.13 mg/ml. Of the 13 children who were cyclophosphamide resistant, 10 attained CR and 2 were in PR. Of the 4 children who were cyclosporine resistant, 2 attained CR, 1 was in PR while 1 child required discontinuation of therapy as he developed HUS. The mean urine spot Pr/Cr ratios were significantly lower (0.33 ± 0.58 vs. 13.5 ± 21.9, p = 0.002) and the mean serum albumin levels were significantly higher (3.92 ± 0.35 vs. 2.39 ± 0.56, p = 0.00005), as compared to those prior to TAC therapy. Of the 16 children who attained CR, 2 patients are off steroids and TAC and in sustained remission, while the rest 14 are still on TAC therapy. The mean duration of follow-up is 290 ± 126 days.

The most common side effect was watery diarrhoea, which was seen in 7 of the 22 children. This resolved with a decrease in the dose of TAC in 6 children, while 1 child developed recurrent diarrhoea and was unable to achieve target TAC levels. Acute renal dysfunction was observed in 3 children; it resolved with a decrease in dose in 2 children while one child developed TAC-induced HUS and required discontinuation of therapy. The mean eGFR in these children at the time of last follow-up was similar to that prior to initiation of TAC therapy (97.9 ± 21.2 vs. 96.4 ± 18.4, p = 0.30). Hyperglycaemia was seen in 2 of the 22 children; it improved with a decrease in dose in one, while in the other child TAC had to be withdrawn. Of the 17 children, 5 were on concomitant antihypertensive therapy.
In 2 children, there was an increase in the dosage of anti-hypertensive agents following the initiation of TAC therapy.

Discussion

This is the largest study so far on the safety and efficacy of TAC therapy in children with SRNS. This was a pilot study to evaluate the efficacy of TAC, which was not funded by any pharmaceutical company. In our experience, of the 19 children who received adequate therapy, 16 (84%) patients attained a CR and another 2 (10.5%) had a PR. These remission rates are much better than the average remission rate of 60–65% that have been reported with other immunosuppressive agents in SRNS [1]. Cyclosporine A (CsA) has been reported to be beneficial in SRNS [3]. MMF has also been used in children with nephrotic syndrome but it has been found to be beneficial in frequent relapsing and steroid-dependent children. There is anecdotal experience with MMF in children with SRNS with poor results. The available data on the efficacy of TAC in NS is scant and contradictory. There are anecdotal reports suggesting its beneficial effect in SRNS [11–13]. In the largest study till date, Loeffler et al. [14] observed a CR rate of 81%. However, this series was retrospective, i.e. it included children with IgA nephropathy besides MCD and FSGS. It had a mix of children with SDNS, and only 5 had SRNS [14]. In contrast, in another recent study, TAC was not found to be beneficial in 10 children with SDNS [15]. Our study is prospective, homogenous and includes children only with SRNS secondary to MCD, DMH and FSGS. Although this was not a controlled trial, 13 of these 22 children had received prior therapy with cyclophosphamide and another 4 had received CsA without any benefit. Of the 13 children who had been treated with cyclophosphamide, 10 attained a CR and 2 were in PR, with an overall remission rate of 92% in the cyclophosphamide-resistant subgroup. An interesting observation in our study was that of the 4 children who were nonresponsive to CsA previously, 2 achieved CR and 1 was in PR with TAC. The overall remission rate was 75% in this difficult-to-treat subgroup. Thus, in our study, TAC was found to be beneficial in SRNS children who were nonresponsive to other treatments, including CsA. Our experience is similar to that of Loeffler et al. [14] who too reported that TAC was beneficial in CsA nonresponsive patients [14].

The mechanisms behind the efficacy of TAC in SRNS are not precisely known. There is some data to suggest that TAC has differing effects on proteinuria in NS compared with cyclosporine [20]. Maruyama et al. [21] also demonstrated better inhibition of the vascular permeability factor cultured from patients suffering from MCD with TAC than with cyclosporine. TAC also has better cytokine suppression than cyclosporine, which may also influence the differing responses to therapy. Calcineurin inhibitors exert their effects by binding to proteins called immunophilins. The predominant immunophilin of TAC is FK-506-binding protein 12 (FKBP 12) in T cells. The complex of TAC and FKBP 12 inhibits calcineurin phosphatase, an essential enzyme for the activation of nuclear factor of activated T cells (NF-AT). NF-AT is an essential transcription factor for the transcription of cytokine genes in T cells. Thus, TAC inhibits the transcription of T cell cytokines like IL-2 and IFN-γ. The calcineurin–TAC complex is not completely specific for NF-AT and can also interfere with other substrates including IkB, NA-K-ATPase and nitric oxide synthetase [10]. Besides its impact on IL-2, it was shown that TAC decreases mRNA levels of IL-3, IL-4, GM-CSF, TNF, IFN and c-myc in activated human peripheral blood T cells. Thus, TAC affects growth and differentiation of T- and B-cell lymphocytes resulting in potent immunosuppression.

The therapy was tolerated in most of the patients. The most common side effect was diarrhoea. This resolved by decreasing the dose of TAC except in one patient who was unable to achieve target levels due to diarrhoea. As he continued to have persistent proteinuria, the TAC therapy was stopped. This side effect is well described with TAC therapy but has not been reported previously in the two previous reports [14,15]. Another child developed HUS following TAC therapy—the drug was stopped and he was also withdrawn from the study protocol. Two of the 22 children in our study developed hyperglycaemia and one required discontinuation of therapy. In the study by Sinha et al. [15], 1 of the 10 children developed hyperglycaemia. This underscores the need for regular blood sugar monitoring in these children. Of the 22 children, 2 developed hypertension requiring additional antihypertensive therapy.

This is the largest study so far on the safety and efficacy of TAC therapy in children with SRNS. Our results suggest that TAC is an effective therapeutic modality for children with SRNS, including the subgroup of children nonresponsive to the current therapeutic modalities like cyclophosphamide and cyclosporine. However, TAC had to be withdrawn in three children in our study because of its side effects. Hence, these children require close monitoring. Our results support the need for a larger multi-centric trial comparing the efficacy of TAC with cyclosporin in children with SRNS.

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References


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