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A prospective trial of safety and efficacy of low-dose tacrolimus therapy in steroid resistant nephrotic syndrome

Avinandan Banerjee, Smita Subhash Divyaveer*, Praveen Malvade, Tanima Das Bhattacharya, Chetan Mahajan, Vaibhav Tiwari, Arpita Raychaudhury, Dipankar Sircar, Sanjay Dasgupta, Arunansu Bandyopadhyay, Debabrata Sen, Rajendra Pandey

Department of Nephrology, Institute of Post Graduate Medical Education & Research, Seth Sukhlal Karnani Memorial Hospital, Kolkata, India

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ABSTRACT

Introduction: A Significant proportion of steroid-resistant nephrotic syndrome (SRNS) patients who do not achieve remission will progress to end-stage renal disease (ESRD).
Objectives: Calcineurin inhibitors (CNIs) are recommended as a first line therapy in SRNS but the data on tacrolimus (TAC) and its potential nephrotoxicity in SRNS patients is limited.
Patients and Methods: This is a prospective single arm study conducted at IPGMER Kolkata from August 2013 to December 2015. All SRNS patients underwent kidney biopsy before the initiation of therapy. Patients with identified secondary causes of FSGS, eGFR ≤ 45 mL/min/1.73 m^2, or more than 5% of interstitial fibrosis and tubular atrophy (IFTA) on biopsy were excluded. TAC was given 0.075 mg/kg (adjusted to maintain TAC trough level i.e. T0 of 5-7 ng/mL) with low-dose steroids. Those who completed 12 months of TAC underwent second biopsy. Primary outcome was a percent of partial or complete remission (CR) or refractory. Secondary outcome was time to achieve remission, relapses, and proportion of patients who had adverse effects.
Results: Thirty-two patients were enrolled. Overall remission was seen in 28 patients (87.5%). CR was seen in 17 (53.13%) and partial remission (PR) was seen in 11 (34.38%). Four patients (12.5%) were refractory to therapy. Average time to achieve PR was 72.53 ± 62.57days while average time to achieve CR was 63.84 ± 27.32 days. Mean TAC dose required was 1.75 ±0.86 mg. Thirteen patients (40.63%) had relapses. One patient needed admission for diarrhea. All other adverse effects were managed on outdoor basis. None required discontinuation of TAC therapy. Compared with the baseline biopsy two patients had increase in IFTA and another one developed IFTA on one year protocol biopsy.
Conclusion: Low dose TAC maintaining trough levels (T0) of 5 to 7 ng/mL with low dose steroid is an effective option for patients with SRNS. It is well tolerated and efficacious in achieving remission.

Implication for health policy/practice/research/medical education:
Treatment of steroid resistant nephrotic syndrome (SRNS) is challenging. Tacrolimus (TAC) has been found effective in previous trials however its potential side effects of acute and chronic nephrotoxicity continues to be a concern. In this study we targeted a lower dose range of TAC. Only one patient developed reversible acute kidney injury which improved by lowering the dose. Protocol biopsies at one year showed a small percentage of patients having increased/new onset chronicity. As a comparator group was not available it remains difficult to ascertain if this was due to TAC or the disease per se. However, it is likely that lower dose range of TAC would result in lesser long-term toxicity while being equally efficacious as the higher/wide dose range.
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Introduction

The annual incidence of nephrotic syndrome (NS) is estimated to be 2 to 7 per 100 000 children and epidemiological evidence suggests higher incidence from South Asia (1). About 10% of idiopathic NS children have/develop steroid resistance (2). An increasing incidence of steroid-resistant NS has been reported over the last few decades (3,4). SRNS is associated with 50% risk for end-

***Corresponding author**: Smita Subhash Divyaveer, Email:divyaveer.ss@gmail.com

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stage renal disease (ESRD) within five years of diagnosis if patients do not achieve a partial or complete remission (CR) (5). Calcineurin inhibitors (CNIs) have been tried with good success in achieving remission in SRNS and are recommended as first line agents for SRNS but data with tacrolimus (TAC) is limited (3,6). The effectiveness of CNI in SRNS may be due to its direct effect on podocytes independent of its immunosuppressive action (7). From the available literature on kidney transplantation, TAC is preferable to cyclosporine regarding potency, cosmetic adverse effects, pharmacokinetic properties as its absorption which is not bile dependent and probably less incidence of hypertension and dyslipidemia (8,9). There is evidence to suggest response to TAC is better than cyclosporine with respect to proportion of relapses during therapy. TAC is additionally efficacious in cyclosporine unresponsive/dependent patients (10,11). The acute and chronic nephrotoxicity of TAC continues to be an important concern (12). The available literature on toxicity of CNI is mainly from the studies in kidney transplant recipients but these may not be applicable to SRNS patients directly where the target trough levels (T0) used in studies have been variable/lower (7,13).

Objectives

We planned this prospective single arm study to analyze the efficacy and safety of TAC in children with idiopathic SRNS while maintaining a lower trough level (T0) than that used in previous studies. As serum creatinine (sCr) is not an accurate measure of TAC toxicity until significant histologic damage has already occurred, we did a protocol biopsy in all the patients who completed 12 months of TAC therapy. Very few prospective studies have described protocol biopsies in SRNS. The beneficial effect of angiotensin-converting enzyme inhibitors in SRNS is well established hence, ramipril was given to all patients as supportive therapy along with statins when indicated (3).

Patients and Methods

Study population

This is a single center prospective single arm trial conducted at IPGMER Kolkata from August 2013 to December 2015. Institutional ethics committee approval was taken and children of either gender between 1 to 18 years of age diagnosed with SRNS whose parents/guardians gave written informed consent for TAC therapy, protocol biopsy and regular follow up were screened. All patients underwent renal biopsy with ultrasound guidance with 18 gauge gun and 2 cores were taken, one each for light microscopy and immunofluorescence staining for IgG, IgA, IgM, C3, C1q, kappa and lambda light chains prior to initiation of therapy and work up for secondary causes including serological evaluation for hepatitis B surface antigen (HBsAg), hepatitis C virus (Anti HCV), human immunodeficiency virus (HIV-ELISA), Complement levels (C3, C4), ANA by immunofluorescence. Those with identified secondary causes,estimated glomerular filtration rate (eGFR) by Schwartz formula ≤45 mL/min/1.73 m^2, family history of NS, membranous nephropathy, membranoproliferative glomerulonephritis or > 5% interstitial fibrosis and tubular atrophy (IFTA) on biopsy were excluded.

Patients who were eligible according to the study criteria were started on TAC 0.075 mg/kg/d divided into two doses 12 hours apart. TAC dose was increased or decreased to maintain trough levels (T0) at 5-7 ng/mL. Details of the therapy have been given as supplementary data. All patients enrolled in the study who received TAC for one year underwent second renal biopsy (protocol biopsy) at one year. For the second renal biopsy, only light microscopy was performed.

Outcomes

Primary outcomes; proportion of patients who achieved complete remission (CR), partial remission (PR) or refractory. Secondary outcomes; time to achieve remission, relapses, proportion of patients who had adverse effects.

Ethical issues

The research followed the tenets of the Declaration of Helsinki; 2) informed consent was obtained, and 3) the research was approved by the institutional ethical committee of IPGME&R, Research Oversight Committee, Institute of Post Graduate Medical Education & Research, Kolkata, India.

Statistical analysis

Statistical analysis was performed using SPSS statistics (version 17.0). The results were expressed as means ± standard deviation (SD). Comparisons were made with the chi-square test and Fisher's exact test, for categorical variables as applicable, and Wilcoxon signed-rank test for continuous variables and P value ≤0.05 was considered to be statistically significant.

Results

During the study period 34 patients were diagnosed with SRNS after exclusion as per above criteria. One patient died due to sepsis prior to initiation of TAC and one patient was lost to follow up after 1 month, hence 32 patients were included in analysis. Mean duration of follow up was 17 ± 4.53 months.

The average age at enrollment and that at diagnosis of NS and SRNS is shown in Table 1. Twenty patients were less than 12 years of age and 12 were between 12 to 18 years of age. Female patients had slightly earlier age of onset compared to male patients.

Primary steroid resistance was found in 18 patients (56.25%) and secondary was found in 14 (43.75%). 10 (31.25%) of the 32 patients in the study had hypertension. None of the patients required antihypertensive other than ACEI/ARB for control of hypertension. Microscopic hematuria was found in 11 (34.38%). Minimal change disease (MCD) was the major histologic finding and was found in 23 patients

Table 1. Average age at onset of NS, diagnosis of SRNS and age at enrollment ± SD (in months)

	Male (n = 11)	Female (n = 21)	Overall (n = 32)
Age at enrollment (months)	112.4±23.89	101.08±46.52	104.21± 67.75
Age at onset of NS (months)	85.7 ± 38.32	83.96 ± 33.32	84.11 ±56.02
Age at diagnosis of SRNS (months)			100.66 ± 43.32

(71.88%) and remaining 9 (28.13%) had focal segmental glomerulosclerosis (FSGS). 11(34.38%) patients received prior immunosuppression other than steroids. Of them seven patients had received cyclophosphamide and four received levamisole.

Overall response rate i.e. partial and CR considered together was seen in 28 patients (87.5%), of which CR was found in 17 patients (53.13%) and PR in 11 patients (34.38%). Resistant cases were 4 (12.5%). The response to therapy in the two histologic groups i.e. MCD and FSGS are shown in Figure 1. The difference in response between MCD and FSGS was not statistically significant ($P = 1.000$). Secondary SRNS has a better overall response rate (92.36%) compared to primary SRNS which had overall response rate of 83.33% as shown in Figure 2. However, this was not statistically significant ($P = 0.621$). Of the 7 patients treated for SRNS, who were also resistant to cyclophosphamide (prior immunosuppression) two had CR and the other five patients attained PR. Three patients who had received levamisole earlier attained CR with TAC therapy.

Average time to reach at least a PR (in those who did not achieve CR during entire follow up) was 72.53 ± 62.57 days while the average time to achieve CR was 63.84 ± 27.32 days. Average time to achieve PR in patients who attained a CR eventually was 44.77 ± 36.58 days. Mean TAC dose required was 1.75 ± 0.86 mg/kg (0.085 ± 0.028 mg/kg) minimum being 1.25 mg/d and maximum needed was 3.5 mg/d. Mean T0 level of the patients was 5.744 ± 1.46 ng/mL.

Thirteen (40.63%) patients had relapses during the study period. Total episodes of relapses were 16, of which 13 occurred on tapering therapy and three occurred during therapy while on initial TAC therapy. Thirteen episodes of relapses were associated with nephrotic range proteinuria while 3 of them had sub-nephrotic proteinuria. T0 levels during relapses were in the range 3.1-5.4 ng/mL. Five relapses were triggered by an upper respiratory tract infection, one on stopping steroid, for the others no specific trigger was identified. All the patients who had nephrotic relapse on tapering therapy were treated with 2 mg/kg of steroids and increasing the TAC dose to the previous level. All of them attained CR and continued to be in CR till last follow up with TAC at previous dose and steroid tapered slowly to 0.25 mg/kg/alternate day. Patients who had sub-nephrotic relapse, were treated with increasing their TAC dose (to achieve T0 close to the upper limit of target range). Each of these attained CR and remained to be in CR till last follow up. Average time to first relapse was

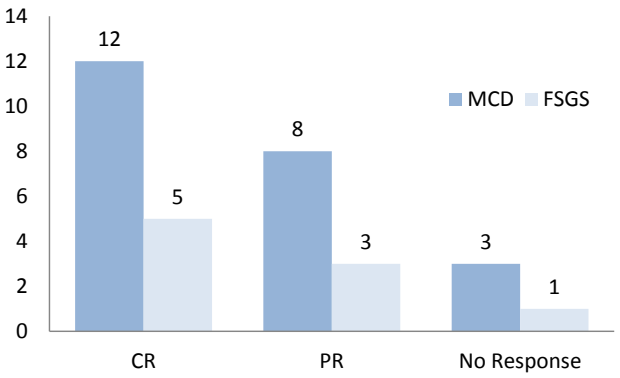

Figure 1. Response to therapy in two histologic groups (n).

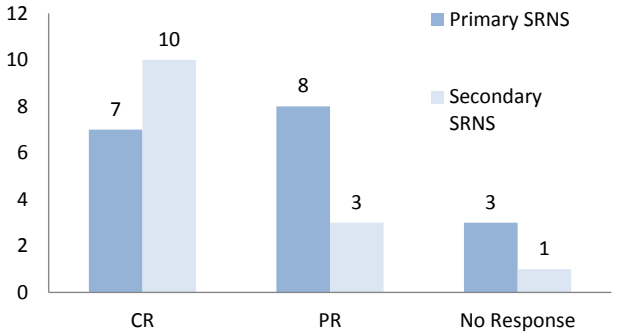

Figure 2. Response to therapy in two clinical subgroups (n).

Table 2. Adverse effects of steroid therapy at the time of enrollment and those related to Tac therapy (parenthesis indicate the % of patients)

Steroid	Tacrolimus
Growth retardation (29.4)	Diarrhea (11.76)
Hypertension (29.4)	Hypertension (5.88)
Striae (47.05)	Tremors (11.76)
Cushingoid features (58.82)	Fungal infection (11.76)
Diabetes mellitus/impaired glucose tolerance (0)	Acute kidney injury (3.125)

15.72 ± 4.64 months. Duration of relapse was 26.65 ± 6.24 days and the average time to remission after relapse was 28.66 ± 15.36 days. The adverse effects of steroid therapy at the time of enrollment and those related to TAC therapy are shown in Table 2. Only one patient had acute kidney injury (AKI) but it resolved after decreasing the TAC dose. Only one patient required admission for diarrhea. All other adverse effects were managed on outdoor basis. None of the patients required discontinuation of TAC

Table 3. Comparison of efficacy of Tac in SRNS in our study with other studies

	This study	Gulati et al (14)	Butani et al (15)	Roberti and Vyas (17)	Choudhry et al (10)	Bhimma et al (20)
Proportion of patients on TAC included in analysis	32	19	16	19	18	20 (FSGS SRNS only)
Proportion of primary SRNS	18 /32 (56.25%)	19/22	12/16			
Proportion of secondary SRNS	14 /32 (43.75%)	3/22	4/16			
CR	53.13%	84%	94%	58%	42.8%	40%
PR	34.38%	10.5%		32%	42.8%	45%
CR+PR	87.5%			81%	85.7%	
No remission	12.5%	5.2%	6.25%	9%	14.3%	15%
Time (days/weeks) to any remission (CR/PR)	Mean: 72.53 ± 62.57 d	Mean: 63.2 ± 44 d	Median: 29 d	Median: 8 wk		
Time to achieve CR	Mean: 63.84 ± 27.32 d		Median: 120 d			
Target T0 (ng/mL)	5 to 7	5 to 10	5 to 10			7 to 15
Mean T0 level	5.744± 1.46	9.54 ± 5.13	Range 1.8-12.3			
Duration of follow up	25±11.53 mon	290 ± 126 d	Median: 22 mon	Mean: 55 mon		
% of patients who had relapses	40.63%		47%			

therapy. No patient had thromboembolism and none developed diabetes mellitus/impaired glucose tolerance or new onset hypertension during the course of the study. One patient required increasing dose of ramipril to control hypertension.

Mean serum creatinine (sCr) at the beginning of the study of the patients was 0.78 ± 0.19 mg/dL with a range of 0.33-0.98 mg/dL. While at 12 months follow up mean sCr was 0.71 ± 0.25 mg/dL with a range of 0.42-0.96 mg/dL. The difference of mean sCr at 0 and 12 months was not statistically significant ($P = 0.326$). Mean eGFR at the beginning was 105.16 ± 36.58 mL/min with a range of 61.25 to 161.31 mL/min. Mean eGFR at 12 months follow up was 107.03 ± 44.25 mLmin with a range of 67.06 to 139.70 mL/min. The difference of mean eGFR at 0 and 12 months was not statistically significant ($P = 0.288$). The urinary ACR and serum albumin at presentation and after 12 months follow up declined and increased respectively, each with P value of 0.001. Average number of glomeruli on pre-treatment biopsy was 21 ± 8.32 number. Average proportion of glomeruli on protocol biopsy was 18.2 ± 7.84 number. On initial biopsy, one patient with MCD had three globally sclerotic glomeruli out of 21 and one patient with FSGS had one globally sclerotic glomerulus out of 24. Two patients had five percent IFTA on initial biopsy which increased to 15 to 20% on protocol biopsy at one year. One patient whose initial biopsy had no IFTA, subsequently had increase in IFTA to 10 percent (same patient also had 4 globally sclerotic glomeruli on repeat biopsy). The proportion of MCD and FSGS were the same even on repeat biopsies. The vascular compartment was normal in the pre and post-treatment biopsies.

Discussion

In our study most patients were females. The gender distribution described in the previous studies of SRNS has been variable; some with male (14,15) and others with female (16,17) predominance. Like the previous studies regarding the administration of TAC in SRNS, more patients in our study had primary steroid resistance than secondary steroid resistance. The histologic diagnosis was found to be either MCD or FSGS in this study with MCD being more than twice as common as FSGS. We have excluded patients with MPGN and MN in our study. This is in contrast to most previous studies which have reported near equal or higher incidence of FSGS compared to that of MCD with small proportion of cases with MPGN, MN, C1q nephropathy, and IgA nephropathy (12-15). The difference could possibly be due to fact that FSGS is a focal pathology which may be missed on biopsy, however, none of patients who had MCD in the first biopsy showed FSGS in the protocol biopsy. Hence the difference may be reflective of the actual difference in incidence between the two entities.

As shown in Table 3, the response to TAC in our studies was comparable to that reported earlier. We were able to attain similar remission rates with a lower TAC dose and a predefined narrow range of target trough level for TAC. We found that patients, who attain a CR, achieved a PR earlier than the time taken to achieve PR in patients who attained only a PR (i.e. no CR during the entire follow up). Hence, an early response may be indicative of an eventual CR. In our study the mean urine spot Pr/Cr ratios were significantly lower and the mean serum albumin levels were significantly higher at the end of study as compared to the values at enrollment. This was also similar to the findings of other studies.

Average time to first relapse after initiation of TAC therapy was 15.72 ± 4.64 months. All the patients whose dose was tapered had a relapse while 9.38% relapsed in spite of not

having any change in their TAC dose. The concept of a longer treatment period for children with FSGS–SRNS was proposed by Hamasaki et al after observing a 100% rate of late relapses following the cessation of cyclosporine therapy in such patients. The authors suggested a minimum of 24 months of therapy (18).

The mean eGFR in our study at the end of 12 months was similar to the eGFR prior to initiation of TAC, similar to that found by Gulati et al (14) and Butani et al (15). However, Hsu et al reviewed limitations in the use of sCr for GFR measurements and noted, failure to detect small decrements in renal function (19). Hence we included protocol biopsy of patients who completed TAC therapy for one year in our study design. Two patients had increase in IFTA from 5 to 15%-20% and one patient who had no IFTA in initial biopsy had 10% IFTA on second biopsy. There was no stripped fibrosis. There was no significant change in arterioles. Both of these patients were diagnosed as FSGS on initial biopsy and both are in CR till last follow up. The proportion of patients were very small to be statistically compared with baseline. The study design did not include control group. Hence, the possibility of progression of underlying disease contributing to IFTA cannot be completely ruled out though the patients were clinically in remission at 12 months. Data of biopsy-proven chronic calcineurin inhibitor toxicity in SRNS is very scarce. A previous study by Bhimma et al, reported that in 14 patients who had not achieved remission after 12 months of FK506 therapy, no evidence of CNI toxicity was detected (20). Another retrospective study found significant increase in IFTA in sequential biopsies while on TAC therapy. However this study had some limitations like prior administration of cyclosporine, lack of a control group and probability of sampling error (21).

No genetic testing was conducted in the patients. While, there is some evidence to suggest that even those patients with identified genetic abnormalities, can be responsive to TAC therapy possibly due to a direct effect on podocytes (7,15).

As there was no control group, we cannot comment on the economic impact the low-dose TAC therapy would have. However, it is likely to be of benefit as the complications of nephrotic state would be reduced and the proportion of hospital visits and admissions would be lesser. It is likely to have a huge impact on quality of life too. Longer studies with larger sample size of patients and protocol biopsies are needed further to confirm the efficacy and toxicity of low-dose TAC to assess its impact on hard outcomes in SRNS.

Conclusion

In children with SRNS, TAC therapy is an effective therapy. TAC associated nephrotoxicity is a concern in these children who often require long-term TAC and this nephrotoxicity can be minimized using low-dose TAC while achieving remission rates comparable to that observed in previous studies which had used higher doses. The overall response rates are similar in MCD and FSGS. The overall response may be better in secondary SRNS than primary SRNS patients. The ideal duration of therapy and tapering of TAC doses needs to be established in further studies.

Limitations of the study

The major limitations of this study are small proportion of patients, no parallel group comparison and relatively short follow up. In case of the two patients who did not respond to TAC, we did not attempt using higher doses of TAC and it could not be determined if higher doses would have been helpful.

Authors' contribution

AB and SSD were involved in care of patients, data collection, procedure of renal biopsy, statistical analysis and the primary manuscript preparation. PM detailed the initial framework of the study and began acquisition of data and was involved in patient care. TDB, CM and VT were in patients' care and data acquisition. AR, DiS, SD, ArB, DeS, and RP guided the study and provided help with clinical decision making throughout the study. RP has been a mentor and also provided critical insights while manuscript preparation. All authors read, revised, and approved the final manuscript.

Conflicts of interest

There were no points of conflicts to declare.

Ethical considerations

Ethical issues (including plagiarism, data fabrication, double publication) have been completely observed by the authors.

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