Renal interstitial fibrosis in children treated with FK506 for nephrotic syndrome

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Abstract

Background. Steroid-dependent, steroid-resistant or frequently relapsing nephrotic syndrome carries a poor prognosis, including progression to renal failure. There are a number of studies confirming the efficacy of FK506 in steroid-resistant or steroid-dependent nephrotic syndrome. Although the use of this medication is becoming more common, we know very little about the potential nephrotoxicity when used in nephrotic syndrome.

Method. We retrospectively reviewed the characteristics and biopsy findings of 11 children with steroid-dependent or frequently relapsing nephrotic syndrome treated with FK506. Two sequential biopsies were evaluated for the change in interstitial fibrosis, measured by a quantitative stereological method, and the change in arteriolar hyaline thickening, tubular atrophy and interstitial fibrosis, graded according to Banff criteria.

Results. There was an increase in interstitial fibrosis (P = 0.005), with a median absolute change in the per cent volume density between initial and follow-up biopsies of 1.8% [interquartile range (IQR) 3.9%]. Median percentage change in volume density of interstitial fibrosis, relative to volume density of interstitial fibrosis prior to initiating FK506, was 93% (IQR 138%). Banff scores for interstitial fibrosis and tubular atrophy also increased following tacrolimus therapy (P = 0.04 for both). Average FK506 trough level over the treatment period was significantly associated with change in fibrosis (Spearman’s rho = 0.67 and P = 0.02).

Conclusions. This is some of the first histological data concerning tacrolimus nephrotoxicity in childhood nephrotic syndrome. Although the role of the natural progression of the underlying disease in the observed change is not definitively clear, the changes seen are in keeping with the known nephrotoxic effects of FK506 demonstrated in renal transplant. This increase is small when presented as a median change. However, there were a number of children who had a larger change in fibrosis. The factors predictive of interstitial fibrosis while on FK506 are not well defined; the findings from this study suggest that FK506 level may be a factor. Given the observations and limitations of the few published studies, there is an obvious need for further study in a large multicenter prospective trial.

Keywords: calcineurin toxicity; FK506; FSGS; interstitial fibrosis; treatment-resistant nephrotic syndrome

Introduction

Steroid-resistant, steroid-dependent or frequently relapsing nephrotic syndrome, with focal segmental glomerulosclerosis (FSGS) as the most common entity in these categories, is forms of nephrotic syndrome associated with the development of end-stage renal failure [1]. Despite years of research, the pathophysiology and clinical management still remain an enigma. Children with these treatment patterns have a poor prognosis; in addition to the high risk of progression to renal failure, they are at high risk of complications including infection, hypercoagulability and malnutrition [2, 3]. These children also experience serious side effects from continuous steroid therapy. Therapeutic success using current strategies is suboptimal, with the majority of therapies reporting <50% efficacy [4].

FK506 is an alternate treatment modality for steroid-resistant or steroid-dependent nephrotic syndrome [5–8]. A pilot trial of FK506 in steroid-resistant nephrotic syndrome by McCauley et al. [5] was the first report of seven patients who all responded positively to FK506 despite being resistant to prior therapies. FK506 has been used at our center in patients with notable success. The results of our experience with 17 patients have recently been published and demonstrated a complete remission rate of 81% and a partial remission rate of 13% (totaling 94%) in children treated with FK506 for steroid-resistant or steroid-dependent nephrotic syndrome [8]. Over the last several years, there have been a number of studies confirming the efficacy of FK506 in steroid-resistant or steroid-dependent nephrotic syndrome in children [9–11].

Despite its recognized efficacy, there is still limited data on the renal histological changes that occur with such therapy. Histological nephrotoxicity of FK506 has been well...
established in the solid organ transplant population, where this drug has become one of the mainstays of immunosuppressive therapy [12, 13]. Such nephrotoxicity is histologically characterized by arteriopathy and tubulointerstitial atrophy and fibrosis [14]. As we are currently continuing to treat steroid-resistant and steroid-dependent childhood nephrotic syndrome for an increasing duration of time with FK506, information concerning nephrotoxicity in this population of children is critical in decision making regarding treatment options. A multicentered study in adults with idiopathic membranous nephropathy receiving FK506 found no sign of calcineurin inhibitor toxicity in a subgroup of six patients, who underwent serial renal biopsies after at least 1 year of therapy [15]. A recent study by Butani and Ramsamooj [10] described the findings from serial biopsies obtained from seven children with steroid-resistant nephrotic syndrome receiving FK506 after median treatment duration of 24 months; two children showed an increase in interstitial fibrosis and tubular atrophy, one of which also had increased staining for transforming growth factor beta (TGF-β). Bhimma et al. [16] evaluated serial biopsies in 14 children with FSGS who had not achieved remission after 6 months of FK506 therapy and reported no evidence of calcineurin inhibitor toxicity in any.

We report on the renal biopsy changes children treated with FK506 for steroid-dependent or steroid-resistant nephrotic syndrome.

Materials and methods

We retrospectively reviewed the characteristics and biopsy findings of 11 children with steroid-dependent or frequently relapsing nephrotic syndrome treated with FK506. The study received full approval by the University of Alberta Human Research Ethics Board. Children were included in the study if they had a diagnosis of steroid-dependent or frequently relapsing disease and had undergone renal biopsy prior to initiation of FK506 therapy in addition to biopsy following a variable period of treatment with the drug. All previous immunosuppressive agents (with the exception of prednisone) had been discontinued prior to the initiation of FK506. Children who relapsed while on FK506 were treated with standard dosing of prednisone (60 mg/m²) until remission followed by a gradual taper over 2 months.

Renal biopsies were analyzed by a renal pathologist (B.S.) in a blinded fashion. The primary outcome measure was the change in interstitial fibrosis between initial and follow-up biopsies. Interstitial fibrosis was assessed in Masson’s trichrome-stained sections using a quantitative stereological analysis. Digital images were obtained from the Masson’s trichrome-stained sections using a light microscope (Eclipse E200; Nikon, Tokyo, Japan) at a magnification of ×20, by a digital video camera (Nikon Coolpix, Tokyo, Japan). Stereological analysis was performed on 10 digital images per case using a point counting method [17–19]. A Photoshop® system (Adobe Photoshop 5.5) was used to create an orthogonal grid with 546 points composed of 21 horizontal and 26 vertical test lines. The area related to one grid unit was 625 × 100 μm² with the grid line length of 250 μm. The volume density (Vv) of interstitial fibrosis per renal cortex (Vv = VPF/VP) was estimated by counting the number of points that hit interstitial fibrosis (VPF) and renal cortex (VP). In addition, arteriolar hyaline thickening (ah), tubular atrophy (ct) and interstitial fibrosis (ci) were semi-quantitatively graded according to the Banff criteria [20].

Data were collected on baseline characteristics as well as potential clinical predictors of change in interstitial fibrosis. These include age, sex, diagnosis, disease duration prior to first study biopsy, time between biopsies, duration of FK506 therapy, per cent of time between biopsies on FK506, average FK506 trough level, average FK506 per kilogram per day, cumulative FK506 exposure (dose per kilogram), use of an angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker, hypertension and number of relapses. Average FK506 level was determined by calculating the mathematical average of all FK506 levels drawn during the treatment period under the study. FK506 levels at the extremes of the range for the individual were reviewed and, if confirmed as false levels by repeat testing, were excluded. Cumulative FK506 exposure in dose per kilogram was determined by reviewing all the individual patient’s weights during the period of FK506 therapy under study and dividing the period into intervals based on stable weight. If the weight changed by 1 kilogram, a new interval was defined. The cumulative dose per kilogram for each interval was determined based on the weight for that interval and the total cumulative dose per kilogram determined by adding all the intervals together.

Glomerular filtration rate (GFR) was estimated according to the Schwartz formula before and after FK506 therapy. The rate of change in estimated glomerular filtration rate (eGFR) during the period on FK506 was also determined. Serial serum creatinine levels measured throughout the period of treatment on FK506 were recorded and used to determine eGFR. Schwartz formula for estimating GFR is given by the equation, GFR = k × ht/Scr, where k is a constant, ht is the height of the child (centimeter) and Scr is the serum creatinine concentration (μmol/L) [21]. In the Schwartz equation, the constant k used for adolescent males (≥13 years of age) was 62 and for all other children in this study, the constant was 49 [21]. To determine the rate of change in GFR for each individual, linear regression was applied to all the serial estimations of GFR for that individual to determine a slope of change in GFR per month. All children had at least five estimations of GFR by which slope was calculated. Measurements of creatinine during acute nephrosis were excluded from this determination.

Statistical analysis

Stata 9.2 for Windows was used to conduct the statistical analysis. Wilcoxon signed-rank test was used to assess change in interstitial fibrosis and Banff scores over the follow-up interval, as well as the change in GFR. To determine the association between potential predictors and the outcome measure, Spearman’s rank correlation was used for continuous measures and Mann–Whitney U-test was used for dichotomous measures. P-value <0.05 was considered as significant in all analyses.

Results

Baseline characteristics of the study sample and the characteristics of FK506 therapy are shown in Table 1. In total, 11 children met the inclusion criteria, 9 of whom were males. Nine children had a histological diagnosis of FSGS, with the other two children having a histological diagnosis of IgM and C1Q nephropathy, respectively. Mean (and median) age at the time of the first study biopsy was 8 years (range 2–13 years of age). Six children (55%) had hypertension, all of whom were on either an angiotensin-converting enzyme inhibitor or an angiotensin II receptor blocker. These children were all hypertensive at the time of first study biopsy, prior to initiation of FK506; they continued to be managed as hypertensive throughout the study interval. One additional child who was normotensive was also on an angiotensin-converting enzyme inhibitor.

All children had received cyclosporine A prior to initiation of FK506 therapy. The median cumulative cyclosporine dose, daily cyclosporine dose and time on cyclosporine were 130.2 g/kg [interquartile range (IQR) 257.2], 4.2 mg/kg/day (IQR 2.6) and 31 months (IQR 60), respectively. Three of the 11 patients reported had cyclosporine discontinued and FK506 started concurrently with the first study biopsy. The remaining patients were on cyclosporine for a variable period after first study biopsy. At the time of first study biopsy, the median disease duration was 24 months (IQR 64.2 months). Median time between paired biopsies used in analysis was 34 months (IQR 58 months), and median duration of FK506 therapy between biopsies was 19 months (IQR 17 months). The median per cent of time between biopsies on FK506 was 58 per cent (IQR 53%).
Mean average FK506 level for the group was 7.9 μg/L [median (range) 7.9 (4.4–11.2) μg/L]. Mean daily FK506 dose was 0.17 mg/kg/day [median (range) 0.18 (0.10–0.20) mg/kg/day]. Median cumulative FK506 dose was 106 mg/kg (IQR 77 mg/kg).

Indications for follow-up renal biopsies are given in Table 2. In 8 of the 11 children, the second renal biopsy was performed at the discretion of the treating physician after a period of FK506 therapy solely to look for the possibility of FK506 nephrotoxicity. Results are illustrated in Figure 1 and summarized in Table 2. The median percentage change in volume density of interstitial fibrosis prior to initiating FK506, was 93% (IQR 138%). There was a significant increase in the volume density of interstitial fibrosis following FK506 therapy (P = 0.03 for both absolute change and per cent change). There was also a significant change in Banff scores for interstitial fibrosis and tubular atrophy following FK506 therapy (P = 0.04 for both). There was no significant change in arteriolar hyaline thickening (ah scores) between sequential biopsies.

Average FK506 trough level over the treatment period was significantly associated with change in fibrosis, with Spearman’s rho = 0.67 and P = 0.02. Average daily FK506 dose per kilogram was also significantly associated with change in fibrosis, with Spearman’s rho = 0.62 and P = 0.04.

### Table 1. Baseline characteristics of the study sample and characteristics of FK506 therapy

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>HTNa</th>
<th>ACEI or ARBb</th>
<th>Disease duration (months)</th>
<th>Biopsy interval (months)</th>
<th>Time on FK506 (months)</th>
<th>% of interval on FK506</th>
<th>Mean FK506 level (μg/L)</th>
<th>FK506 dose (mg/kg/day)</th>
<th>Total FK506 (mg/kg)d</th>
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<td>M</td>
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<td>No</td>
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<td>83</td>
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</tbody>
</table>

*aHypertension.*

*bAngiotensin-converting enzyme inhibitor or angiotensin II receptor blocker.*

*cDisease duration at time of first study biopsy.*

*dCumulative dose of FK506 in the interval between paired biopsies.*

### Table 2. Proteinuria and renal histology before and after FK506 therapy; IF, Interstitial fibrosis

<table>
<thead>
<tr>
<th>Patient</th>
<th>UPCRa</th>
<th>ah scoreb</th>
<th>ci scoreb</th>
<th>ct scoreb</th>
<th>% IFc</th>
<th>Absolute Δ in % IF</th>
<th>Relative Δ in % IF</th>
<th>Indication for second biopsy</th>
</tr>
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<tr>
<td>1</td>
<td>649</td>
<td>9</td>
<td>0</td>
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<td>3.79</td>
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<td>12</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.30</td>
<td>13.33</td>
<td>Protocold</td>
</tr>
<tr>
<td>3</td>
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<td>68</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1.60</td>
<td>2.80</td>
<td>74.99</td>
</tr>
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<td>608</td>
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<td>1</td>
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<td>4.40</td>
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<td>1</td>
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<td>2.90</td>
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<td>3.31</td>
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<td>1</td>
<td>1</td>
<td>4.65</td>
<td>9.00</td>
<td>93.54</td>
</tr>
</tbody>
</table>

*aUPCR, urine protein/creatinine ratio; pre, value at start date of FK506; post, value at time of second biopsy.*

*bArteriolar hyaline thickening (ah), tubular atrophy (ct) and interstitial fibrosis (ci) by Banff criteria.*

*cPer cent volume density of interstitial fibrosis.*

*dRenal biopsy done at the discretion of the treating physician to look for possible FK506 nephrotoxicity (no clinical indication).*
no significant correlation observed between the interstitial fibrosis and the urine protein/creatinine either prior to or following FK506 therapy. We evaluated if any measures of cyclosporine exposure predicted the change in fibrosis; duration of therapy, average cyclosporine level, percent of biopsy interval on cyclosporine, cumulative dose per kilogram and average daily dose per kilogram were not associated with the change in fibrosis seen.

The eGFR before and after FK506 therapy were not significantly different ($P = 0.89$) by nonparametric methods for the study group as a whole. Nevertheless, some children appeared to have a decline in their eGFR, while others had an increasing slope as illustrated by Figure 2. The change in interstitial fibrosis did not correlate with this change in eGFR over time. The only predictor variable with significant correlation with the individual’s eGFR over time was the number of months on FK506 ($\rho = 0.75$ and $P = 0.007$).

**Discussion**

This is some of the first histological data concerning FK506 nephrotoxicity in childhood nephrotic syndrome. The increase in interstitial fibrosis and tubular atrophy seen in children on FK506 therapy is in keeping with the known nephrotoxic effects of FK506 demonstrated in renal transplant. This increase is small when presented as a median change. However, as illustrated in Figure 1, there were a number of children in this study who appeared to have a larger change in fibrosis.

Current evidence suggests that increased expression of fibrogenic molecules such as TGF-β is involved in the pathogenesis of FK506 nephrotoxicity [22]. It has been shown in both animal models and isolated cell culture of mesangial cells that FK506 induces expression of TGF-β at both the messenger RNA and protein level and this expression is dose dependent [23, 24]. In our study, greater increases in interstitial fibrosis associated with higher mean FK506 level during treatment could potentially represent the clinical impact of such a dose-dependent mechanism. The ability of angiotensin-converting enzyme inhibitors and angiotensin II receptor blockers to suppress TGF-β expression and reduce fibrosis is well recognized; we were unable to demonstrate a relationship between the use of an angiotensin-converting enzyme inhibitor or angiotensin II receptor blocker and the degree of interstitial fibrosis. Again, our study has a small number of subjects and thus is likely underpowered to detect a true association between these predictor variables and the outcome if one exists. This warrants further investigation.

In the study by Butani and Ramsamooj [10], they found no statistically significant increase in interstitial fibrosis in serial biopsies in a cohort of seven children with a similar clinical presentation to our population. FK506 level, cumulative exposure and daily dose of FK506 were similar to the present study. They did, however, find that in the cohort with worsening fibrosis compared to those with no histological changes, the average inter-biopsy FK506 level was higher. We confirmed this finding with statistical significance. The finding in our study that change in fibrosis also correlates with daily FK506 dose likely represents the same association.

The study by Chen et al. [15] which looked at an adult population with idiopathic nephrotic syndrome (membranous) did not show a statistically significant increase in interstitial fibrosis following FK506 therapy; however, the trough FK506 level achieved beyond 6 months of therapy was lower than in our study. The population was new-onset nephrotic syndrome with no prior immunosuppressive treatment and five of the six subjects that underwent follow-up renal biopsy had not achieved complete remission with FK506 and had ongoing active disease. This provides some evidence, although significantly limited, that the small change in fibrosis seen in our study may not be due to natural progression of the underlying disease over the timeframe studied. In addition, it provides support to the hypothesis that FK506 trough level may be an important predictor of interstitial fibrosis in the treatment of nephrotic syndrome with FK506.

We were unable to demonstrate a correlation between duration of FK506 therapy and change in interstitial fibrosis, which was surprising, and may be due to the small size of the sample studied. The previous study by Bhimma et al. [16] reported that in 14 patients who had not achieved remission after 12 months of FK506 therapy, there was no evidence of

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**Fig. 1.** Interstitial fibrosis pre- and post-FK506 use.

**Fig. 2.** Change in glomerular filtration rate over interval of FK506 therapy.
calcineurin inhibitor toxicity. No data are provided about the methodology of calcineurin inhibitor toxicity measurement or quantification. Furthermore, all patients were on low-dose oral prednisone and an angiotensin-converting enzyme inhibitor throughout the FK506 treatment period. The discrepancy between their results and ours may be due to a longer duration of FK506 before follow-up renal biopsy in our study or alternatively the different use of concurrent medications.

The results of this study may be confounded by disease severity and the change in interstitial fibrosis may represent the natural history of disease; however, we found no significant correlation between the number of nephrotic relapses (which included those preceding FK506 therapy), the duration of disease prior to initiating FK506, the time from disease onset to first or second study biopsy or the biopsy interval and the change in interstitial fibrosis. Degree of proteinuria before or after FK506 therapy was also not predictive of fibrosis. A significant limitation of this study is that there was no control group in which changes in interstitial fibrosis not related to FK506 could be assessed. Current standard of care does not include repeat renal biopsy for a child who is clinically stable and not on a calcineurin inhibitor. Also as a standard of care, children only receive FK506 at our center after failing cyclosporine therapy. Hence, for the current observational study, there is no control population that is appropriate for comparison. Comparison to a population with steroid-dependent or steroid-resistant disease on cyclosporine would have limited use except in the setting of a randomized trial in which patients were at similar stages in their course of disease and therapy.

All 11 children in this study had received cyclosporine prior to the initiation of FK506 therapy which may contribute to the development of interstitial fibrosis in these children. However, we were unable to show any significant correlation between the measures of cyclosporine exposure and the outcomes of interest. This may be due to the small number of patients observed in this study. The biopsy interval also did not correlate with the change in fibrosis, which may suggest that the total duration of calcineurin inhibitor exposure is not as important as the drug level achieved, particularly in light of the positive findings related to FK506 level and daily dose.

Clinical outcomes, including blood pressure, change in degree of proteinuria and achievement of remission, relapses on FK506 and side effects, for the majority of the children reported in this case series have been previously reported [8]. In the current study, we found no change in eGFR for the group when taken together before and after FK506 therapy. As shown in Figure 2, there were a number of children who had a decrease in eGFR over time. Serum creatinine is the most commonly used method of estimating GFR in children [21]. Despite its ubiquitous use, creatinine is less than ideal as a marker of GFR because its appearance in urine is a result of both glomerular filtration and tubular secretion. As a result, with decreasing renal function, estimates of GFR can overestimate actual GFR by 10–20% [25]. Hsu et al. [26] reviewed limitations in the use of creatinine for GFR measurements and noted that in addition to propensity to overestimate kidney function and failure to detect small decrements in function, individual variation of eGFR determinations based on creatinine are influenced by age, gender, muscle mass, race, hydration status, physical activity and creatinine analysis technique. It is therefore possible that any decrement in eGFR, albeit small, went undetected in this analysis due to limitations of creatinine as a measure of kidney function. While Hsu et al. [26] acknowledged that iothalamate and insulin are better measures of GFR, these are not necessarily as accessible or economical on a clinical basis, particular in the context of a retrospective study. In future prospective trials assessing FK506 toxicity in steroid-sensitive or steroid resistant nephrotic syndrome, specific measures of GFR using more precise methods are required to address the issue of detectable changes in renal function related to therapy.

The use of protocol biopsies in the motoring of therapy toxicity and disease progression has merit in the context of nephrotic syndrome. Most protocol biopsy data derive from renal transplant literature, where such studies have assisted in the characterization of subclinical rejection and calcineurin toxicity [27]. Although protocol biopsies are limited by sampling error, expense and patient morbidity, they do provide a useful characterization of disease progression not otherwise detectable with clinical parameters. We employed protocol biopsies in a cohort of patients with steroid-dependent or frequently relapsing nephrotic syndrome with the goal of demonstrating the effect of therapy toxicity independent of disease progression; we showed increased interstitial fibrosis potentially related to therapy and not otherwise detectable clinically. Such data are useful in assisting with modification of treatment to preserve renal function in the long term.

Despite the limitations of this study, it provides some early information about the histological changes that occur in children with steroid-dependent or frequently relapsing nephrotic syndrome treated with FK506 following cyclosporine therapy. Although the results are confounded by the possible effects of cyclosporin and the natural progression of disease, it does suggest possible factors predictive of the change in fibrosis in this population, which need to be rigorously tested in larger prospective studies. Although there was a statistically significant change in interstitial fibrosis between paired biopsies in these children treated with FK506, the median absolute difference was only 1.8%. It is our opinion that the demonstrated success rate of FK506 in inducing remission in children with steroid-resistant or steroid-dependent nephrotic syndrome likely outweighs the risk of progression in interstitial fibrosis that may be due to FK506 therapy. The factors predictive of interstitial fibrosis while on FK506 are not well defined. The findings from this study add to the previous study of a smaller cohort [10] in suggesting the lowest possible dose of FK506 to maintain remission be used in children with steroid-dependent or steroid-resistant nephrotic syndrome. Given the observations and limitations of the few published studies, there is an obvious need for further study in a large multicenter prospective trial.

Conflict of interest statement. None declared.

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