Leflunomide for BKvirus: Report of Seven Kidney-Transplanted Children

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ABSTRACT

Background: Leflunomide is an immunosuppressive agent commercialized for treatment of rheumatoid arthritis. Because of its immunosuppressive and possible antiviral properties, leflunomide has been evaluated in some case series of BKVAN with favorable results, mostly in adult patients. Leflunomide targeted levels are usually between 50 and 100 mg/L in kidney transplant adult patients. Data in pediatric population are scarce.

Objective: To assess the effect of leflunomide on BKvirus in kidney-transplanted children.

Methods: Therapeutic drug monitoring of leflunomide is routinely performed by measuring its active metabolite, teriflunomide, using a simple HPLC-UV method. Pediatric kidney transplant patients with at least one teriflunomide sample between 2010 and 2017 were retrospectively included in this study. Viremia control was defined as undetectable BK viremia or a decrease of more than 1 log in the viral load from the baseline after two months of treatment. Adverse events were recorded.

Results: A total of 7 patients from 3 centers was included. 6 were only kidney transplant recipients; 1 was a lung-kidney transplant recipient with cystic fibrosis. All patients reported high load BK viremia but none developed BKVAN. For 67% of the patients, complete BK viral clearance was observed during leflunomide treatment with drastic immunosuppressive therapy reduction. Mycophenolate was indeed discontinued in almost all patients. Of note, leflunomide concentrations were significantly higher when viremia was controlled. Only 33% of the observed concentrations were >40 mg/L. The patient with cystic fibrosis had lower concentrations with higher drug doses. No hepatotoxicity was observed in this study and no patient experienced graft rejection. Leflunomide was suspected to cause hemolytic anemia and one patient experienced biological pancreatitis.

Conclusion: This study evidenced the wide interindividual variability of the exposure and supported the routine practice of leflunomide with a suggested target level of 30–40 mg/L in pediatric kidney transplanted patient. However, because of the very limited number of patients in our series, further investigations are needed to validate this suggestion.

KEYWORDS: Leflunomide; Kidney transplantation; Pediatrics; BK virus; Cystic fibrosis; Mycophenolate

INTRODUCTION

Primary BK infection takes place in early childhood, most often without symptoms, and remains dormant in the urinary tract and possibly in lymphocytes and the brain [1, 2]. BKV replication can be reactivated following immunosuppressive therapy associated with transplantation, and reducing the amount of immunosuppression is the treatment strategy of choice. A 30%–50% cut in immunosuppressive therapy dosage has successfully eliminated the virus in some studies conducted in adults [3, 4].

Leflunomide is an immunosuppressive agent commercialized for treatment of rheumatoid

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arthritis. The drug active metabolite, teriflunomide, can inhibit the phosphorylation of PDK1 and Akt, two sites of mTOR, in a dose-dependent manner, and reduce BK large T antigen expression and BK DNA replication [5, 6]. Because of its immunosuppressive and possible antiviral properties, leflunomide has been evaluated in some case series of BKVAN with favorable results, mostly in adult patients [7].

Teriflunomide has a long elimination half-life of approximately two weeks in patients, which is most likely due to enterohepatic circulation and biliary recycling [8], reaching a steady state after approximately 20 weeks [9]. Long half-life allows better compliance, especially in young patients. Plasma or blood concentrations of teriflunomide are commonly monitored because of high leflunomide doses used, known inter-individual pharmacokinetic variability, and hepatotoxicity or hematotoxicity risks [10]. The leflunomide targeted levels are usually between 50 and 100 mg/L in kidney transplant adult patients [11]. Data in pediatrics population are scarce.

The primary objective of this study was to evaluate BK clearance by leflunomide therapy in seven pediatric patients with kidney transplantation and to evaluate leflunomide tolerance. The secondary objective was to describe the amount of cut in dosage in immunosuppressive therapy and to compare the dose and concentrations in our pediatric patients compared with those of in adults.

PATIENTS AND METHODS

Therapeutic drug monitoring of leflunomide is routinely performed in our center dosing teriflunomide using a simple high performance liquid chromatography with an ultraviolet detector (HPLC-UV).

All kidney-transplant patients, aged between 0 and 18 years with at least one teriflunomide concentration measured between 2010 and 2017, were retrospectively included in this study. For each patient, data were collected on demography (sex, height, weight, age), date of kidney transplantation, and leflunomide treatment (the initiation and discontinuation dates, amount). The treatment duration was calculated according to the start and stop dates (when stop date was unavailable, the last date with dosage available was considered the stop date). We also collected data on the combined immunosuppressive agents (dose reduction), BK infection (PCR and biopsy results), and adverse events with leflunomide as the suspected drug.

Viremia control was defined as undetectable BK viremia or a decrease of more than 1 log in the viral load from the baseline after two months of treatment. The dosage of drug was adapted according to the targeted level in adults.

The mean concentration of teriflunomide was compared in groups with different levels of viremia control and tolerance (adverse event observed by clinicians) using Student's t test for independent samples. Descriptive statistics were calculated with MS Excel and R *ver* 3.2.3. Correlation coefficients were measured using Pearson, Spearman or Kendall tests depending on the data distribution (R *ver* 3.2.3). Comparison between concentrations or doses were performed using student's t test for independent samples or Welch test, depending on the comparison of the ratio of their variances (R *ver* 3.2.3).

RESULTS

A total of seven patients, with a mean age of 12.1 (range: 6–18) years, from three centers were included (Table 1). Patient #5 was a lung transplant patient with cystic fibrosis (CF). The median number of available teriflunomide concentrations per patient was 25.4 (range: 3–93). All patients reported BK viremia but no BKVAN was observed.

Immunosuppression was reduced by an average of 92% in mycophenolate and 46% in tacrolimus doses in six patients. Mycophenolate was discontinued in five of the six patients,

Table 1: Characsitics of studied patients

Patient	Age	Sex	Weight (kg)	Date of kidney transplantation	Introduction of leflunomide post-transplantation (m)	Average dose (mg/day)	Average dose (mg/kg/day)	Average concentration (mg/L)	Variability on concentration vs dose ratio	BK viremia evolution at the end of the study	Time post leflunomide (m)	Viremia control (-1 log after 2 months of Leflu)	Treatment duration (m)	Adverse event
1	7	F	20.5	18/04/2013	18	33.3±10.3 (n=6)	1.6±0.5 (n=6)	37.4±17.8 (n=6)	37%	+1.6 log	3	N	>5	—
2	14	F	30	20/02/2013	7	54.5±9.0 (n=33)	1.5±0.1 (n=33)	36.3±15.5 (n=43)	49%	-3.2 log	21	Y	17	—
3	6	M	25.4	14/06/2011	36	26.7±11.5 (n=3)	1.0±0.5 (n=3)	55.0±34.6 (n=3)	20%	Undetectable	1	Y	6	Mouth ulcers
4	10	M	21	24/04/2012	3	35.0±16.9 (n=8)	1.7±0.8 (n=8)	20.2±15.5 (n=10)	49%	-1.8 log	26	Y	>18	—
5*	14	M	23	19/07/2009 and 04/11/2014	14	67.1±31.3 (n=42)	2.3±1.1 (n=42)	28.3±17.1 (n=93)	34%	Undetectable (but nephrectomy)	31	N	>45	Hemolytic anemia and 50% dose reduction, then increase without recurrence
6	16	F	55.2	19/06/2011	5	20.0±0.0 (n=11)	0.4±0.0 (n=11)	53.0±8.5 (n=13)	18%	No data	—	—	11	biological but clinically asymptomatic pancreatitis and Leflu withdrawal
7	18	F	60	11/09/2009	10	40.0±0.0 (n=10)	0.7±0.0 (n=10)	34.0±5.7 (n=10)	17%	Undetectable	5	Y	>12	—
All	12.1± 4.6 (n=7)	4F/ 3M	34.0± 16.8 (n=7)	—	13.3	50.8±25.8 (n=113)	1.6±1.0 (n=113)	32.7±17.7 (n=178)	122%	—	—	—	>16.3	—

*Patient with cystic fibrosis

and reduced by 50% in other patients. After reduction, the mean tacrolimus dose was 0.14 mg/kg daily.

Leflunomide was started at a median dose of 1 mg/kg and secondary adapted to reach a through level in 70% of the patients, mostly by increasing doses to up to 2 mg/kg in four of five patients. A total of 33% of the observed concentrations was >40 mg/L (n=6); 18% of the concentrations were >50 mg/L (n=5). A total of 31% under-expositions (<20 mg/L) were recorded in four patients. Because patient #5 had CF, lower concentrations were observed (Table 1).

For patient #1, BK viremia was increasing but renal biopsy was not in favor of BK virus infection three months after the initiation of leflunomide. In patient #5, BK PCR was undetectable only after nephrectomy; he was then categorized in the group of no viremia (control). Leflunomide concentrations were significantly higher when viremia was controlled (34.4±16.8 ng/mL [n=4] vs. 28.9±17.2 ng/mL [n=2], p=0.0434). No patient experienced graft rejection.

No hepatotoxicity was observed in our study. One episode of hemolytic anemia was observed in patient #5; leflunomide was reduced by 50%, but it was increased again after the adverse event resolved without recurrence of the anemia. Patient #3 reported mouth ulcers. Leflunomide was discontinued in patient #6 due to biological, but clinically asymptomatic, pancreatitis. It was also discontinued in two patients because of a switch to everolimus. Leflunomide concentrations were not significantly different when an adverse event occurred (32.0±18.9 ng/mL [n=3] vs. 33.7±15.5 ng/mL [n=4]). Some patients complained about the difficulty of swallowing pills.

DISCUSSION

Pape and colleagues investigated the current practice from 90 European physicians [12] and found that reducing immunosuppression is considered the first step in treating significant BK infection. Successful BK clearance, using mean dose reduction by 44% and 41% in mycophenolate and tacrolimus, respectively, was observed without addition of an antiviral therapy [3]. Usually, the first immunosuppressant to be discontinued is mycophenolate (75%), as this treatment is indeed associated with an increased incidence of BK viruria compared with treatment with everolimus or cyclosporine [13]. Leflunomide was added by 25% of the physicians [12].

In our patients, an average of 92% cut in dosage of mycophenolate and 46% of tacrolimus was observed. Because patient #5 received lung transplant and had CF, immunosuppression was only reduced by 33%. This patient received a higher dosage of leflunomide (mean±SD of 2.3±1.1 mg/kg/day).

The use of leflunomide in pediatric patients for this indication had been previously reported by Araya, *et al*, in three children [14]. The age of the patients and dosage of the drug were comparable to our studied patients (13.7±5.7 yrs vs. 12.1±4.6 yrs, p=0.572; and 33.3±5.8 vs. 39.4±16.0 mg/day, p=0.554). The overall average leflunomide concentrations they reported were also consistent with our results (37.3±11.1 mg/L vs. 37.7±12.5 mg/L in our population, p=0.956). Leflunomide was well tolerated and used for more than two years [14].

If we compare the data with those obtained in adults [7, 15], no significant difference was observed for leflunomide concentrations. The dosage administered to reach these through level is higher in children than in adults (1.62 mg/kg in our patients vs. 20 mg in adults, translating into a mean dose of 0.30 mg/kg for a 70 kg adult), which illustrates the usual higher clearance observed in children. In our patients, despite the high dosage used, only 33% of the observed concentrations were >40 mg/L; 18% of the concentrations were in the target range for TDM performed in adults. The daily leflunomide dose required was at least 60 mg for 40% of our patients, which is lower than the recent study of Nesselhauf, *et al*, where 60% of the adult patients required

almost 60 mg of the drug [16]. Only two patients received 20 mg of the drug, which is the recommended maintenance dose in rheumatoid polyarthritis and psoriasic rheumatism.

Total BK viral clearance observed during leflunomide therapy was associated with a drastic immunosuppressive therapy reduction in 67% of our patients. In our patients, teriflunomide concentrations were significantly higher when viremia was controlled (n=4). However, pharmacodynamic analysis revealed no association between teriflunomide concentrations and reduction in BK virus PCR results in 52 adult patients [17]. Further studies are needed to discuss this inconsistency.

Regarding the safety of the drug, we observed side effects in three of seven patients. One patient reported mouth ulcers; since he was not treated with mTOR, it was attributed to leflunomide. Oral ulcers are reported in 3%–5% of leflunomide-medicated patients with rheumatoid arthritis who develop adverse events; withdrawal of leflunomide for a month results in <50% healing [18]. For another patient, leflunomide was suspected to cause hemolytic anemia. The dosage of the drug was reduced by 50%. However, secondary increase in the dose did not cause anemia. Finally, a 16-year-old female patient experienced biological pancreatitis after 11 months of leflunomide therapy. Leflunomide was indeed reported to be associated with pancreas toxicity [19]. In our patient, the average leflunomide concentration was as high as 53 mg/L, even though the dose administered in this patient was only 20 mg, which illustrated the interindividual variability of the exposure and supports routine TDM for this drug. According to Kiely, *et al*, there is no relationship between the plasma concentration of teriflunomide and adverse events [20]. In our patients, leflunomide concentrations were not significantly different when an adverse event occurred. However, this series included a CF patient. After exclusion of CF patient from the analysis, the mean leflunomide concentration was significantly higher when an adverse event occurred (53.3±14.8 ng/mL vs. 33.7±15.5 ng/mL, p<0.0001).

In conclusion, for 67% of our pediatric patients, a drastic decrease in immunosuppressive treatment and leflunomide was associated with BK viral clearance. Immunosuppression was reduced by an average 92% cut in dosage in mycophenolate and 48% in tacrolimus, without any impacts on the graft loss. Of note, leflunomide concentrations were significantly higher when viremia was controlled. This study evidenced the wide interindividual variability of the exposure and supported the routine practice of TDM with a suggested target level of 30–40 mg/L in pediatric kidney transplanted patients. However, because of the very limited number of patients included in this study, further investigations are needed to validate this suggestion.

REFERENCES

1. Müller A, Beck B, Theilemann K, *et al.* Detection of Polyomavirus BK and JC in Children With Kidney Diseases and Renal Transplant Recipients. *Pediatr Infect Dis J* 2005;**24**:778-81.
2. Rinaldo CH, Tylden GD, Sharma BN. The human polyomavirus BK (BKPyV): Virological background and clinical implications. *Apmis* 2013;**121**:728-45.
3. Saad ER, Bresnahan BA, Cohen EP, *et al.* Successful treatment of BK viremia using reduction in immunosuppression without antiviral therapy. *Transplantation* 2008;**85**:850-4.
4. Huang G, Wang CX, Zhang L, *et al.* Monitoring of polyomavirus BK replication and impact of preemptive immunosuppression reduction in renal-transplant recipients in China: A 5-year single-center analysis. *Diagn Microbiol Infect Dis* 2015;**81**:21-6.
5. Liacini A, Seamone ME, Muruve DA, Tibbles LA. Anti-BK virus mechanisms of sirolimus and leflunomide alone and in combination: toward a new therapy for BK virus infection. *Transplantation* 2010;**90**:1450-7.
6. Bazin C. Leflunomide an Immunosuppressive Drug for Antiviral Purpose in Treatment for BK Virus-Associated Nephropathy After Kidney Transplantation. Antivir Drugs - Asp Clin Use Recent Adv. 2012. Available from *www.intechopen.com/books/an* Accessed July 15, 2018.
7. Teschner S, Gerke P, Geyer M, *et al.* Leflunomide Therapy for Polyomavirus-Induced Allograft Nephropathy: Efficient BK Virus Elimination Without Increased Risk of Rejection. *Transplant Proc* 2009;**41**:2533-8.
8. Alcorn N, Saunders S, Madhok R. Benefit-Risk Assessment of Leflunomide Arthritis 10 Years After

Licensing. *Assessment* 2009;**32**:1123-34.

9. Rozman B. Clinical Pharmacokinetics of Leflunomide. *Clin Pharmacokinet* 2002;**41**:421-30.
10. Ng JC, Leung M, Wright AJ, Ensom MH. Clinical Pharmacokinetic Monitoring of Leflunomide in Renal Transplant Recipients with BK Virus Reactivation - A Review of the Literature.*Clin Pharmacokinet* 2017;**56**:1015-31.
11. Josephson MA, Gillen D, Javaid B, *et al.* Treatment of renal allograft polyoma BK virus infection with leflunomide. *Transplantation* 2006;**81**:704-10.
12. Pape L, Tönshoff B, Hirsch HH. Perception, diagnosis and management of BK polyomavirus replication and disease in paediatric kidney transplant recipients in Europe. *Nephrol Dial Transplant* 2016;**31**:842-7.
13. van Doesum W, Gard L, Bemelman F, *et al.* Incidence and outcome of BK polyomavirus infection in a multicenter randomized controlled trial with renal transplant patients receiving cyclosporine-, mycophenolate sodium-, or everolimus-based low-dose immunosuppressive therapy. *Transpl Infect Dis* 2017;**19**. doi: 10.1111/tid.12687.
14. Araya CE, Garin EH, Neiberger RE, Dharnidharka VR. Leflunomide therapy for BK virus allograft nephropathy in pediatric and young adult kidney transplant recipients. *Pediatr Transplant* 2010;**14**:145-50.
15. Faguer S, Hirsch HH, Kamar N, *et al.* Leflunomide treatment for polyomavirus BK-associated nephropathy after kidney transplantation. T*ranspl Int* 2007;**20**:962-9.
16. Nesselhauf N, Strutt J, Bastani B. Evaluation of leflunomide for the treatment of BK viremia and biopsy proven BK nephropathy; a single center experience. *J Nephropathol* 2016;**5**:34-7.
17. Krisl JC, Taber DJ, Pilch N, *et al.* Leflunomide efficacy and pharmacodynamics for the treatment of BK viral infection. *Clin J Am Soc Nephrol* 2012;**7**:1003-9.
18. Kalogirou E, Katsoulas N, Tosios K, *et al*. Non-healing tongue ulcer in a rheumatoid arthritis patient medicated with leflunomide. An adverse drug event? *J Clin Exp Dent* 2017;**9**:e325-e328. doi: 10.4317/jced.53428.
19. Douros A, Bronder E, Andersohn F, *et al.* Drug-induced acute pancreatitis: Results from the hospital-based Berlin case-control surveillance study of 102 cases. *Aliment Pharmacol Ther* 2013;**38**:825-34.
20. Kiely PD, Johnson DM. Infliximab and leflunomide combination therapy in rheumatoid arthritis: an open-label study. *Rheumatology* 2002;**41**:631-7.