Pediatr Transplantation 2001: 5: 93–98
Printed in UK. All rights reserved

Pediatric Transplantation
ISSN 1397–3142

Reasons why some children receiving tacrolimus therapy require steroids more than 5 years post liver transplantation

Jain A, Mazariegos G, Kashyap R, Marsh W, Khanna A, Iurlano K, Fung J, Reyes J. Reasons why some children receiving tacrolimus therapy require steroids more than 5 years post liver transplantation. Pediatr Transplantation 2001: 5: 93–98. © Munksgaard, 2001

Abstract: Tacrolimus is a potent immunosuppressive agent and has been used in liver transplantation (LTx) for nearly a decade. More than 70% of children can be maintained on tacrolimus monotherapy, without steroids, by the end of 1 yr post-Tx. This freedom from steroids does not appear to change significantly in subsequent years. The use of steroids has obvious metabolic and cosmetic disadvantages, besides affecting linear growth in children. The present study identifies why some children still require steroid therapy after successful LTx. One hundred and sixty-six consecutive pediatric patients who had undergone primary LTx between October 1989 and December 1992, were included in this study. Follow-up ranged from 6 to 9 yr (mean 7.5±0.8 yr). One hundred and forty-one children were alive in November 1998 and these patients constituted the study group. Their current rate of prednisone use, reason for prednisone use, and prednisone dose were examined retrospectively. Of the 141 patients, 139 (98.5%) had stopped taking steroids at some time-point after LTx. Thirteen patients (9%) were off immunosuppression altogether (group I), 97 were undergoing tacrolimus monotherapy (group II), and the remaining 31 were receiving therapy with steroids and tacrolimus (group III). The mean prednisone dose at the last follow-up was 6.5±4.9 mg/day (median 5.0 mg/day). In group III, two children were never weaned off steroids because of inadequate follow-up (both lived outside the country), and the remaining 29 children completely stopped steroid therapy at some time-point after LTx; however, prednisone was re-introduced for clinically suspected or biopsy-proven rejection in 24. Seven children in group III had completely stopped immunosuppressive therapy either as part of an immunosuppression reduction protocol (n=3) or for suspected or proven post-transplant lymphoproliferative disorder (PTLD) (n=4). In eleven of the 18 children in group III, requirement of steroid for rejection was thought to be related, in part, to non-compliance. In three children in group III, steroids were re-introduced for renal dysfunction, and two of these patients subsequently received a kidney Tx. In one child with cerebral ischemia, steroids were used to reduce brain edema, and another child had features of auto-immune hepatitis. Hence, almost all children can be weaned off steroids when tacrolimus is used as primary immunosuppresson after primary LTx. However, ≈ 22% of children may need re-institution of steroids because of late acute rejection or renal dysfunction. The concomitant use of other non-steroidal immunosuppressive agents with tacrolimus may further reduce the dose and rate of steroid use.

Ashok Jain[1,2], George Mazariegos[1], Randeep Kashyap[1], Wallis Marsh[1], Ajai Khanna[3], Kathy Iurlano[1], John Fung[1] and Jorge Reyes[1]

Thomas E. Starzl Transplantation Institute, [1]Department of Surgery and [2]Department of Pharmaceutical Sciences, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania, [3]Department of Surgery, University of California at San Diego, San Diego, California, USA

Key words: liver transplantation – tacrolimus – steroid – children

Jorge Reyes, M.D., University of Pittsburgh, Thomas E. Starzl Transplantation Institute, Falk Clinic, 4th Floor, 3601 Fifth Avenue, Pittsburgh, PA 15213, USA
Tel.: (412) 692-7867
Fax: (412) 692-6116
E-mail: Reyesj@chplink.chp.edu

Accepted for publication 18 July 2000

This paper was presented at the 25th Annual meeting of American Society of Transplantation in Chicago, May 1999, and at the 5th Congress of International Liver Transplantation Society in Pittsburgh, PA, August 1999.

Abbreviations: ALT, alanine aminotransferase; AST, aspartate aminotransferase; CsA, cyclosporin A; LTx, liver transplantation; LFT, liver function tests; MMF, mycophenolate mofetil; PTLD, post-transplant lymphoproliferative disorder; Tx, transplantation.

The introduction of tacrolimus after LTx has shown a significantly lower rate of acute rejection, steroid-resistant acute rejection, and refractory acute rejection, with a significantly lower concomitant use of steroids when compared with CsA (1–4). We have also shown that nearly 70% of all primary LTx patients undergoing tacrolimus therapy can be weaned off steroids by the end of the first post-transplant year. This rate of steroid-free status was observed to be even higher in infants (age <2 yr) and children (2–18 yr) when compared with adults (18–60 yr) and seniors (age >60 yr) (5), and prompted an active program of weaning immunosuppression in stable LTx patients who are >5 yr post-LTx and >2 yr rejection free with a normal liver biochemical profile (6, 7). Despite this effort, the actual overall freedom from steroids still remained almost the same beyond 5 yr post-Tx, changing little after the first year (8, 9). Steroids have several metabolic, systemic, and cosmetic disadvantages, and an additional adverse impact on growth and development in children. The aim of the present study was to examine all children who were alive beyond 5 yr post-LTx for:

1 the rate of freedom from steroids;
2 the dose of steroid for those who were receiving steroids;
3 the indications for use of steroids; and
4 to examine the liver and renal biochemical profiles in children who were or were not undergoing steroid therapy.

Patients and methods

Between October 1989 and December 1992, 166 children who underwent consecutive primary LTx and received tacrolimus therapy were included in the analysis. Follow-up ranged from 6 to 9 yr (mean 7.5±0.86 yr) until November 1998. Twenty-five children died during the follow-up period, leaving 141 children available for study. The study group consisted of 80 males (56.7%) and 61 females (43.3%); the mean age was 5.4±5.6 yr (median 2.9, range 0.1–17.8). The indications for LTx are shown in Table 1.

Table 1. Indications for liver transplantation (LTx) (n=141)

Indication	n	%
Biliary atresia	70	49.6
Metabolic*	20	14.1
Cryptogenic cirrhosis	10	7.1
Post-necrotic cirrhosis†	8	5.7
Acute fulminant failure‡	8	5.7
Familial cholestasis	5	3.5
Cystic fibrosis	4	2.8
Congenital hepatic fibrosis	4	2.8
Neonatal hepatitis	3	2.1
Secondary biliary cirrhosis	3	2.1
Hepatoblastoma	3	2.1
Budd chiari	1	0.7
Other§	2	1.4
Total	141	

*Alpha-1 anti-trypsin, eight patients; Wilson's disease, five; glycogen storage disease-iv, two; transcarbamylase deficiency, one; cabamyphosphatase synthatase deficiency, one; Crigler Najar, one; oxalosis, one; tyrosinemia, one.
†Hepatitis B virus, one patient; non-A, non-B hepatitis, two; auto-immune hepatitis, three; cytomegalovirus, one; Indian childhood cirrhosis, one.
‡Viral, two patients; tylenol, one; unknown, five.
§Histiocytosis, one patient; nodular regenerative hyperplasia, one patient.

The study population was divided into three groups: group I, children who were completely free from all immunosuppression; group II, children who were undergoing tacrolimus monotherapy; and group III, children who were undergoing therapy with tacrolimus and steroids. Group III was further examined for the dose of steroids and possible reasons for the use of steroids. Our institutional protocol for tacrolimus and steroids has been described previously (2, 5, 10). Steroid tapering starts 2–3 weeks post-Tx. If a child has normal LFT without any prior history of rejection, usually a 25 to 50% decrease in steroid is made over a 2- to 6-week interval. If during this time there is any rise in liver biochemistry, the original dose of steroid is restored and, 4–6 weeks after normalization of the biochemistry profile, another attempt at dose reduction is made. In all patients, the amount and timing of steroid withdrawal was individualized. A rise in the biochemical parameters AST and ALT, with or without a rise in serum bilirubin, in the presence of normal arterial and portal blood

Table 2. Group status and dose of immunosuppression

Group	Status of immunosuppression	Tacrolimus dose (mg/day) mean±SD (median)	Tacrolimus level (ng/mL) mean±SD (median)	Prednisone dose (mg/day) mean±SD (median; range)	n	%
Group I	No immunosuppression	None for mean 66.3±20.4 months (median 65.9; range 26.3–96.3)		None	13	9
Group II	Tacrolimus without steroid (monotherapy)	2.9±2.5 (2.0)	4.9±1.1 (4.9)	None	97	69
Group III	Tacrolimus with steroid	5.9±4.0 (6.0)	8.6±6.8 (8.3)	6.5±4.9 (5.0; 1.25–20)	31	22
Total					141	

Table 3. Reasons for steroid use

Reason for use of steroid	n	% Of children who are on steroid (n=31)*	% Of all children (n=141)
Off immunosuppression and rejection† (PTLD confirmed or suspected n=4, weaning protocol, n=3)	7	22.6	5.0
Monotherapy and rejection/autoimmune process‡	18	58.1	12.8
Renal impairment (kidney transplant, n=2)	3	9.7	2.1
Other causes (suboptimal follow-up, n=2;§ cerebral ischemia, n=1)	3	9.7	2.1
Total	31		

*Twenty-nine children had steroid re-introduced.
†Biopsy confirmed (n=3) or clinically suspected on increased liver function test results (n=4).
‡Biopsy confirmed (n=6: rejection=5, autoimmune process=1) or clinically suspected on increased liver function test results (n=12). §Steroid not withdrawn.

flow and non-dilated biliary tree on ultrasound, was suspected as clinical rejection. When a rise in biochemical parameters was observed of more than three times above the normal range, a liver biopsy was performed. A diagnosis of acute rejection was made according to the criteria described by the International Working Party of Pathologists (11).

Results

The study population was divided into three groups (Table 2). Group I comprised 13 patients (9%) who had been off all immunosuppression for a mean time-period of 66.3±20.4 months (median 65.9, range 26.3–96.3). Of these 13 patients, four had ceased immunosuppression as a part of an immunosuppression weaning protocol (6, 7), four had biopsy-proven PTLD, and five had reactive hyperplasia of lymph nodes without PTLD when immunosuppression was discontinued. Group II comprised 97 children (69%) who were undergoing tacrolimus monotherapy. Group III comprised the remaining 31 children (22%) who were undergoing therapy with tacrolimus and steroids.

A detailed analysis of Group III patients showed that 29 of the 31 children (94%) had stopped steroid therapy at some point after LTx (Table 3). The remaining two children were managed by two local physicians outside the country; therefore no attempt had been made to withdraw steroids as communication was inadequate. Hence, of the 141 children in the study group, 139 (98.6%) had stopped steroid therapy at some point after LTx. However, as described above, 29 of these children (20.9%) required re-institution of steroids and, upon further examination, seven (24.1%) had completely stopped all immunosuppression at some point post-transplant. Three of these seven children were part of a immunosuppression weaning protocol and experienced rejection (6, 7); tacrolimus plus steroids was re-instituted to control the rejection, and all three normalized their liver function. In the other four children, three had biopsy-proven PTLD and one had suspicion of PTLD; therefore, the immunosuppression was discontinued in all four. Subsequently, these four children had biochemical increases in liver function and therefore tacrolimus and prednisone were re-instituted. All four children are now free from PTLD with normal liver function.

Eighteen children (62.1%) were undergoing tacrolimus monotherapy and had increases in their biochemical liver function parameters. Six (33.3%) children had liver biopsy performed: five had a feature of acute cellular rejection, and one had a feature of auto-immune hepatitis. The remaining 12 (66.6%) had clinical suspicion of rejection with biochemical deterioration in liver function. Eleven of these 18 patients (61.1%) were >12 yr (mean age 17.0 yr) and there was some evidence of non-compliance in their immunosuppressive medication. Seven (38.9%) of these 18

Table 4. Liver function and renal function

	Liver function*					Renal function*	
Groups	Total bili. (mg/dL)	ALT (U/L)	AST (U/L)	GGTP (U/L)	Alk. Phos. (U/L)	BUN (mg/dL)	Creatinine (mg/dL)
Group I	0.6±0.3	36±12	30.6±20.2	58±79	261±146	11.3±5.5	0.5±0.25
Group II	0.7±0.5	38±26	37.0±31	47±64	245±342	15.9±8.8	0.7±0.5
Group III	0.7±0.3	47±32	52.0±39	84±90	239±159	16.0±8.3	0.8±0.2

Alk. Phos., alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BUN, blood urea nitrogen; GGTP, gamma glutamyl transferase; Total bili., total bilirubin.
*Mean value.

patients were treated with a steroid bolus (one patient received 1000 mg of methylprednisone and the other five patients received a total of 600 mg of methylprednisone tapered over the subsequent 5 days). Eleven (61.1%) patients were given prednisone 10–20 mg/day. The current mean dose received by these 18 patients was 7.9±5.3 mg/day (median 6.2 mg/day). The mean tacrolimus dose in these 18 children was 2.7± 1.9 mg/day, giving a tacrolimus blood trough level of 5.2±2.5 ng/mL, which was increased to the current dose of 5.9±4.0 mg/day and current tacrolimus blood trough level of 8.6±6.8 ng/mL. The liver function in this group also normalized over time (Table 4).

Three children (10.3%) had impaired renal function. In one child, steroids were re-introduced in order to reduce the dose of tacrolimus. The total leucocyte count of this patient was 3.3 units/ mm^3, hence azathioprine was not considered and MMF was not available for clinical use in January 1994. Two of these three children received a kidney Tx and steroid was re-introduced. Another child suffered ischemic injury to the brain, and steroids were used in an effort to control brain edema. The reasons for steroid use are summarized in Table 3.

Prednisone dose

The dose of prednisone in the 31 children who received it is shown in Table 5. The majority of these children (n=20; 64.5%) were receiving $\leq$5 mg/day prednisone; six (19.6%) were receiving 6–10 mg/day, while only five children (16.1%) was receiving >10 mg/day. Overall, the mean prednisone dose was 6.5±4.9 mg/day (median 5.0; range 1.25–20).

Use of other concomitant immunosuppression agents besides steroids and tacrolimus

Of the 29 children in whom steroids were re-introduced, three were also on azathioprine 12.5–50 mg/day and a further three children were on 1–2 g/day of MMF. Thus, 97 children (68.8%) were receiving monotherapy, 25 (17.7%) were receiving dual therapy with steroids, while only six (4.3%) were receiving triple therapy. Thirteen children (9.2%) had completely stopped immunosuppression.

Liver function and renal function

The mean total bilirubin, AST, ALT, alkaline phosphatase, and gamma glutamyl transferase values (all indicative of liver function), and the blood urea nitrogen and serum creatinine values (both indicative of renal function) of all three groups of children are shown in Table 4. Except for the alkaline phosphatase value, which is partly bone in origin, the other values are within the normal range for all groups.

Table 5. Steroid dose*

Prednisone (mg/day)	No. of children	% Of children who are on steroid (n=31)	% Of all children (n=141)
0	110	–	78
1 to <5	20	64.5	14.2
6 to <10	6	19.4	4.2
>10	5	16.1	3.5
Total	31		

*Mean prednisone dose: 6.5±4.9 mg/day (median 5.0 mg/day).

Rate of hypertension. In group III, three out of 31 children (9.7%) who were undergoing steroid therapy were also receiving anti-hypertensive medications, compared with five out of 110 (4%) from groups I and II combined ($p = 0.28$).

Rate of diabetes mellitus. All children (n=141) were normoglycemic and therefore received no oral hypoglycemic agent or insulin.

Discussion

Our data suggest that almost all children undergoing tacrolimus therapy can be weaned off steroids at some point after primary LTx. However, in $\approx$22% of children, steroids were re-introduced because of a rise in liver function. Steroids were also re-started secondary to renal dysfunction following kidney Tx, and because of adolescent non-compliance and rejection after PTLD.

Makee et al. (12) showed that 71% of children (n=24) could be weaned off steroids successfully 6 months post-LTx. McDiarmid et al. (13) argued in favor of steroid withdrawal for long-term maintenance after LTx. Everson et al. (14) reviewed 16 reports on steroid withdrawal after LTx and concluded that there was no increase in the rate of acute or chronic rejection, with noted benefits in the incidence of hypertension, diabetes, obesity, and cholesterol levels. Abe et al. (15) from Japan has shown successful withdrawal of steroids after living-related LTx in children. Stegall et al. (16), in a prospective conducted trial, reported successful steroid withdrawal 14 days post-LTx in nearly 95% of subjects at 6-months follow-up under therapy with either CsA or tacrolimus plus MMF, with a concomitant benefit in the

incidence of hypertension, diabetes, and hypercholestrolemia.

In our series, no deliberate attempt was made to use some of the newly approved immunosuppressive agents such as MMF or rapamycin. It is quite possible that in the future, more liberal use of azathioprine, MMF, and rapamycin may increase the freedom from steroids in children. With the use of these agents, steroids may need to be used only for a short period of time. There are other reports where MMF has been used successfully to wean children and adults off steroids (16, 17). In a prospective randomized trial of steroid withdrawal in a selected group of 64 patients beyond 1 yr post-LTx under CsA, MMF was found to be safe when used in combination with azathiaprine with improvement in the patients' lipid profile (18). Recently there has been an increasing trend to use these agents at our institution, and this will probably also occur in other centers. In selected patients, Gomez et al. (19) has shown withdrawal of steroids after 1 yr on CsA monotherapy without azathioprine. Padbury et al. (20) reported the successful withdrawal of steroid in 93% of patients (n=168) 3 months post-LTx with only a 34% rate of hypertension. Similarly, Punch et al. (21) reported an 88% success rate in withdrawal of steroid at 1 yr, with subsequent benefits in the incidence of hypertension, weight gain, and cholesterol levels.

It is clear that a greater number of children can be maintained on satisfactory long-term immunosuppression without steroids; however, the role of steroids as initial induction therapy and to treat acute episodes of rejection will remain.

Interestingly, in the present study, some of the children in group III may eventually be weaned off steroids again; conversely, some children from groups I and II may require re-institution of steroids for the reasons stated above. Long-term patient survival hinges on varying degrees of graft acceptance; the variables that impact on this imply host and donor interaction, which may change with intercurrent episodes of infection or noncompliance. In addition, it is important to balance the benefits of monotherapy against the risk of long-term toxicity of such monotherapy. As shown from other reports where a more aggressive policy of steroid withdrawal was implemented, it was not successfully achieved in all cases. Abe et al. (15), from Japan, achieved a 71% rate of steroid withdrawal by 6 months in living-related LTx. Margarit et al. (22), from Spain, showed that 73% of children could be weaned off steroids when treated with CsA. Andrews et al. (23), from Dallas, reported 69% of patients ceasing steroid therapy, with improvement in linear growth. Dunn et al. (24) reported successful withdrawal of steroid in 89% of a selected group of children by 1.5 yr post-transplant. The benefits of freedom from steroids in terms of growth, development, weight loss, hyper-tension, and diabetes, have been described in reports from several centers (15, 18, 20, 23, 24). In this present retrospective study, all children were normoglycemic, and the rate of hypertension was higher in group III compared with groups I and II, but the difference was not significant. Other potential benefits in terms of weight loss, height, and lipid metabolism unfortunately could not be determined in this retrospective study as such information was not available in all children at different post-LTx time-periods.

In conclusion, almost all children can be weaned off steroids after LTx when undergoing tacrolimus therapy; however, ≈22% may need re-introduction of steroids as a result of liver dys-function (a clinical rejection or biopsy-proven late rejection) at some time after complete withdrawal. This rate of re-introduction of steroids can be further reduced by the use of azathioprine, MMF, or rapamycin. With careful selection and close follow-up, up to 9% of children receiving tacrolimus can be weaned off all immunosuppression beyond 5 yr post-LTx, according to the criteria described in our immunosuppressive weaning experience (6, 7).

Acknowledgments

This work was supported in part by research grants from the Veterans Administration, and Project Grant No. DK-29961 from the National Institutes of Health, Bethesda, MD, USA.

References

1. The US Multicenter FK506 Liver Study Group. A comparison of tacrolimus (FK 506) and cyclosporine for immunosuppression in liver transplantation. N Engl J Med 1994: 331: 1110.
2. Jain AB, Fung JJ, Todo S, et al. Incidence and treatment of rejection episodes in primary orthotopic liver transplantation under FK506. Transplant Proc 1991: 23 (1 Part 2): 928.
3. Starzl TE, Donner A, Eliasziw M, et al. Randomised trialomania? The multicentre liver transplant trials of tacrolimus. Lancet 1995: 346: 1346.
4. Anonymous. Randomised trial comparing tacrolimus (FK506) and cyclosporin in prevention of liver allograft rejection. European FK506 Multicentre Liver Study Group. Lancet 1994: 344: 423.
5. Jain AB, Fung JJ, Todo S, et al. One thousand consecutive primary orthotopic liver transplants under FK506: survival and adverse events. Transplant Proc 1995: 27: 1099.
6. Mazariegos GV, Reyes J, Marino I, Flynn B, Fung JJ, Starzl TE. Risks and benefits of weaning immunosuppression in liver transplant recipients: long-term follow-up. Transplant Proc 1997: 29: 1174.
7. Mazariegos GV, Reyes J, Marino IR, et al. Weaning of

immunosuppression in liver transplant recipients. Transplantation 1997: 63: 243.
8. Jain A, Reyes J, Kashyap R, et al. Liver transplantation under tacrolimus in infants, children, adults, and seniors: long-term results, survival, and adverse events in 1000 consecutive patients. Transplant Proc 1998: 30: 1403.
9. Jain A, Reyes J, Kashyap R, et al. What have we learned about primary liver transplantation under tacrolimus immunosuppression? Long-term follow-up of the first 1000 patients. Ann Surg 1999: 230: 441.
10. Jain AB, Mazariegos G, Kashyap R, et al. Comparative long-term evaluation of cyclosporine and tacrolimus in pediatric liver transplantation. Transplantation 2000: 70: 617–625.
11. International working Party. Terminology for hepatic allograft rejection. Hepatology 1995: 22: 648–652.
12. McKee M, Mattei P, Schwarz K, Wise B, Colombani P. Steroid withdrawal in tacrolimus (FK506)-treated pediatric liver transplant recipients. J Pediatr Surg 1997: 32: 973.
13. McDiarmid S. The necessity for steroid induction or long-term maintenance after liver transplantation: the argument against. Transplant Proc 1998: 30: 1449.
14. Everson GT, Trouillot T, Wachs M, et al. Early steroid withdrawal in liver transplantation is safe and beneficial. Liver Transplant Surg 1999: 5 (4 Suppl. 1): S48.
15. Abe M, Fuchinoue S, Koike T, et al. Successful prednisone withdrawal after living-related liver transplantation. Transplant Proc 1998: 30: 1441.
16. Stegall MD, Wachs ME, Everson G, et al. Prednisone withdrawal 14 days after liver transplantation with mycophenolate: a prospective trial of cyclosporine and tacrolimus. Transplantation 1997: 64: 1755.
17. Ringe B, Braun F, Lorf T, et al. Tacrolimus and mycophenolate mofetil in clinical liver transplantation: experience with a steroid-sparing concept. Transplant Proc 1998: 30: 1415.
18. McDiarmid SV, Farmer DA, Goldstein LI, et al. A randomized prospective trial of steroid withdrawal after liver transplantation. Transplantation 1995: 60: 1443.
19. Gomez R, Moreno E, Colina F, et al. Steroid withdrawal is safe and beneficial in stable cyclosporine-treated liver transplant patients. J Hepatol 1998: 28: 150.
20. Padbury RT, Gunson BK, Dousset B, et al. Steroid withdrawal from long-term immunosuppression in liver allograft recipients. Transplantation 1993: 55: 789.
21. Punch JD, Shieck VL, Campbell DA, Bromberg JS, Turcotte JG, Merion RM. Corticosteroid withdrawal after liver transplantation. Surgery 1995: 118: 783.
22. Margarit C, Martinez Ibanez V, Tormo R, Infante D, Iglesias H. Maintenance immunosuppression without steroids in pediatric liver transplantation. Transplant Proc 1989: 21 **((1 Part 2)):** 2230.
23. Andrews WS, Shimaoka S, Sommerauer J, Moore P, Hudgins P. Steroid withdrawal after pediatric liver transplantation. Transplant Proc 1994: 26: 159.
24. Dunn SP, Falkenstein K, Lawrence JP, et al. Monotherapy with cyclosporine for chronic immunosuppression in pediatric liver transplant recipients. Transplantation 1994: 57: 544.