Effect of tacrolimus on survival in hepatitis C–infected patients after liver transplantation

Jacqueline G. O’Leary, MD, MPH, James F. Trotter, MD, Michael A. Neri, Linda W. Jennings, PhD, Greg J. McKenna, MD, Gary L. Davis, MD, and Göran B. Klintmalm, MD, PhD

The observation that cyclosporine inhibits HCV replication in vitro has led some programs to use cyclosporine as the calcineurin inhibitor (CNI) of choice after orthotopic liver transplantation (OLT). Previous studies comparing outcomes with different CNIs used small HCV cohorts or had short-term follow-up. We examined patient survival and fibrosis progression in all HCV-infected adult primary OLT recipients from 1995 to 2004 at the Annette C. and Harold C. Simmons Transplant Institute (n = 516). Patients were categorized by their CNI on day 7 post-OLT, and they were excluded if they died before day 14. Patient and donor age, sex, race, and prevalence of cytomegalovirus infection post-OLT were similar in the tacrolimus and cyclosporine patients. As expected, acute cellular rejection and steroid-resistant rejection were less common in tacrolimus-treated patients. Although no difference in 1-year survival was seen, tacrolimus patients (n = 268) had superior 5-year survival compared to cyclosporine patients (n = 248) (75% vs. 67%; $P = 0.02$). Fibrosis progression was no different between the groups. In our retrospective analysis of 516 post-OLT patients, tacrolimus improved long-term survival compared to cyclosporine in HCV-infected patients, although it did not impact HCV fibrosis progression.

Hepatitis C (HCV) viremia at the time of orthotopic liver transplant (OLT) leads to universal reinfection of the new graft and has been repeatedly shown to decrease graft and patient survival (1–6). When HCV viremia is eradicated pre- or post-OLT, a significant survival advantage is achieved (7). Although HCV infection can be treated with pegylated interferon and ribavirin, sustained virologic response post-OLT is infrequent (8–10). Therefore, transplant professionals often attempt to “optimize” immunosuppression when viral eradication is not possible. Toward this goal, some transplant centers switched their calcineurin inhibitor (CNI) of choice from tacrolimus to cyclosporine when in vitro data showed cyclosporine inhibited HCV viral replication (11–15). In addition, earlier reports raised concern that tacrolimus might reduce the antiviral activity of interferon, although subsequent data did not confirm this observation (16). However, more important than in vitro information on HCV viral replication is how CNIs impact patient survival and HCV fibrosis progression.

To date, only large trials with short-term follow-up or small trials with longer-term follow-up have been published; no large

trial of exclusively HCV-infected patients with 5-year follow-up has been performed to determine if CNI choice affects long-term patient survival and/or fibrosis progression post-OLT (17–23). Toward this goal, we analyzed the survival and fibrosis progression of all our adult primary OLT patients with HCV infection from January 1995 to December 2004 based on CNI assigned by protocol at day 7.

METHODS

Institutional review board approval was granted prior to the initiation of this retrospective study. The Annette C. and Harold C. Simmons Transplant Institute maintains a prospective research database called the Liver Transplant Research Database System, which contains data on all OLT patients since the program’s inception. Cause of death is obtained from death certificates and hospital records and is reviewed by a physician before it is entered into the database. Patients undergo protocol liver biopsies at year 1, 2, and 5; they have additional liver biopsies for abnormal liver function tests. All rejections are diagnosed by biopsy. The METAVIR fibrosis score of all liver biopsies is logged in the database regardless of indication. Pathology results were not rereviewed for the purposes of this study. Compliance with protocol liver biopsies was assessed: the number of patients who obtained a protocol liver biopsy was divided by those who should have obtained a protocol biopsy (defined as patients who were alive without previously meeting the endpoint of stage 3 or 4 fibrosis). Compliance at year 1, 2, and 5 was 88%, 74%, and 73%, respectively.

We examined patient survival and fibrosis progression in all adult primary OLT recipients from 1995 to 2004 (n = 516) who had a diagnosis of HCV and survived longer than 14 days. Patients were categorized by the CNI they received on day 7 post-OLT and excluded from the analysis if they were not on a CNI on day 7.

Patient characteristics were compared using Wilcoxon rank sum, and median values were reported. Patient survival and

From the Baylor Simmons Transplant Institute, Baylor University Medical Center at Dallas.

Corresponding author: Jacqueline G. O’Leary, MD, MPH, Simmons Transplant Institute, Baylor University Medical Center at Dallas, 4610 Worth Street, Dallas, Texas 75246 (e-mail: Jacquelo@BaylorHealth.edu).

Table 1. Patient demographics

Variable	Cyclosporine (n = 248)	Tacrolimus (n = 268)	P value
Age	49	49	NS
MELD at transplant (calculated)	14	16	<0.001
Cold ischemia (hrs)	8.3	7.2	0.003
Male sex	160 (65%)	194 (72%)	NS
Hepatocellular carcinoma	70 (28%)	40 (15%)	<0.001
Donor age	40	39	NS
Race			NS
White	187 (75%)	198 (74%)	
Black	20 (8%)	22 (8%)	
Other	41 (17%)	48 (18%)	
Patients on same CNI at week 4	231 (95%)	248 (93%)	NS
Steroids for immunosuppression on day 7	223 (90%)	221 (82%)	0.02
Antimetabolite			<0.001
Mycophenolate mofetil	63 (25%)	209 (78%)	
Azathioprine	17 (7%)	11 (4%)	
None	168 (68%)	48 (18%)	
Acute cellular rejection	159 (64%)	143 (53%)	0.02
Steroid-resistant rejection	47 (19%)	18 (7%)	<0.001
Cytomegalovirus	54 (22%)	54 (20%)	NS

MELD indicates Model for End-Stage Liver Disease.

fibrosis progression were evaluated by Kaplan-Meier analysis. In addition, Cox proportional hazards analyses were used to assess risk factors for death and fibrosis progression. Stepwise multivariable models were built using factors from the univariate analysis that had a P value <0.1 . The final multivariable models included only factors that were statistically significant. Statistical significance was defined as a $P < 0.05$. SAS 9.1 was used for all statistical analyses.

RESULTS

Table 1 compares the characteristics of patients transplanted for HCV treated with tacrolimus vs. cyclosporine between January 1995 and December 2004, who survived greater than 14 days post-OLT. Patients in both groups had a similar recipient and donor age, were mostly Caucasians of male sex, and had the same likelihood of cytomegalovirus (CMV) infection. In addition, both CNI groups were equally likely to remain on the CNI assigned by protocol on day 7 at week 4. Model for End-Stage Liver Disease (MELD; calculated MELD, not match MELD) at OLT was higher and cold ischemia time was shorter in the tacrolimus-treated patients. Tacrolimus-treated patients were more likely to receive mycophenolate mofetil instead of steroids for chronic immunosuppression and were less likely to experience rejection of any kind than cyclosporine-treated patients.

Figure 1 demonstrates the survival advantage that tacrolimus-treated patients had over cyclosporine-treated patients

Figure 1. Patient survival by calcineurin inhibitor used on day 7 post-OLT in (a) hepatitis C-infected patients and (b) non-hepatitis C-infected patients.

($P = 0.02$). This survival advantage was not appreciated until after year 1. The same survival advantage was also seen in patients without HCV (*Figure 1b*) ($P < 0.001$), although it was appreciated sooner post-OLT.

Kaplan-Meier analysis was undertaken to assess relevant factors associated with survival in the two groups (*Table 2*). Acute cellular rejection, although decreased in incidence in tacrolimus-treated patients, does not explain the survival difference. Tacrolimus-treated patients without steroid-resistant rejection (SRR) had the best survival, and tacrolimus-treated patients with SRR had the worst survival, although SRR was uncommon in tacrolimus-treated patients ($P = 0.007$). All patients, regardless of race, achieved a survival advantage with tacrolimus; however, the survival advantage of tacrolimus treatment was greatest for African American recipients. The absolute survival advantage at 5 years between tacrolimus and cyclosporine was 22% for African Americans vs. 13% for other races and 6% for Caucasians ($P = 0.04$).

Since transplant survival improves when the pretransplant MELD is low (24), we compared the effect of CNI choice on the outcome of patients in different MELD groups (*Table 2*). A MELD of <21 was chosen arbitrarily to represent a low MELD. The survival advantage afforded patients treated with tacrolimus rather than cyclosporine post-OLT was similar to the difference between high and low preoperative MELD score in the cyclosporine-treated patients ($P = 0.002$).

Table 2. Survival for subgroups based on calcineurin inhibitor used

Group	Year		
	1	2	5
Survival based on presence of acute cellular rejection (ACR) ($P = \text{NS}$)			
Cyclosporine: no ACR ($n = 89$)	84%	78%	66%
Tacrolimus: no ACR ($n = 125$)	93%	86%	78%
Cyclosporine: ACR ($n = 159$)	86%	80%	67%
Tacrolimus: ACR ($n = 143$)	88%	83%	73%
Survival based on presence of steroid-resistant rejection (SRR) ($P = 0.007$)			
Cyclosporine: no SRR ($n = 201$)	87%	79%	65%
Tacrolimus: no SRR ($n = 250$)	92%	86%	77%
Cyclosporine: SRR ($n = 47$)	81%	81%	74%
Tacrolimus: SRR ($n = 18$)	72%	61%	56%
Survival by race ($P = 0.04$)			
Cyclosporine: African American ($n = 20$)	90%	70%	55%
Tacrolimus: African American ($n = 22$)	95%	91%	77%
Cyclosporine: white ($n = 187$)	87%	81%	68%
Tacrolimus: white ($n = 198$)	88%	84%	74%
Cyclosporine: other ($n = 41$)	76%	73%	66%
Tacrolimus: other ($n = 48$)	94%	85%	79%
Survival by Model for End-Stage Liver Disease score ($P = 0.002$)			
Cyclosporine: ≥ 21 ($n = 39$)	69%	67%	51%
Tacrolimus: ≥ 21 ($n = 51$)	86%	80%	71%
Cyclosporine: < 21 ($n = 205$)	88%	81%	69%
Tacrolimus: < 21 ($n = 214$)	92%	86%	77%

We analyzed patients' risk of achieving the composite endpoint of fibrosis progression to advanced fibrosis (METAVIR stage 3 or 4) or death from HCV by Kaplan-Meier analysis (Figure 2). The step increases seen in the Kaplan-Meier analysis correspond to protocol liver biopsy time points at years 1, 2, and 5. There was no difference between the groups ($P = \text{NS}$).

Patients with hepatocellular carcinoma were treated under a separate protocol with low-dose cyclosporine and rapamycin, and hepatocellular carcinoma was not evenly distributed between the two CNI groups. Since some of the patients were treated before the Milan criteria were implemented, we performed a separate analysis of patient survival excluding patients who died of recurrent hepatocellular carcinoma, and the survival difference between tacrolimus and cyclosporine remained (data not shown).

We next performed Cox proportional hazards modeling to look for factors that may independently impact post-OLT survival and fibrosis progression (Table 3). Multivariable analysis demonstrated cyclosporine use and preoperative MELD ≥ 21 to be the strongest predictors of decreased post-OLT survival. Since mycophenolate use was colinear with tacrolimus use, this factor was not included in our multivariable analysis. For advanced fibrosis or death from HCV recurrence, multivariable

Figure 2. Fibrosis progression to METAVIR stage 3 or 4 or death from recurrent hepatitis C by calcineurin inhibitor used.

analysis revealed African American race, CMV infection, and acute cellular rejection > 3 months post-OLT as predictive of this composite endpoint. As shown in Figure 2, CNI choice did not impact HCV fibrosis progression.

The primary causes of death are listed in Table 4. Overall, fewer deaths occurred in the tacrolimus-treated group, and therefore each cause of death was represented as a percentage of the total deaths. Recurrent HCV was the most common reason for death in both groups. Malignancy was twice as common in cyclosporine-treated patients as tacrolimus-treated patients; however, cyclosporine-treated patients had 13 deaths from recurrent hepatic malignancies unlike the tacrolimus group, which had 3. Malignancies other than recurrent hepatocellular carcinoma were not different between CNI groups. Of note, 6% of cyclosporine-treated patients died from either acute or chronic rejection compared with none of the tacrolimus-treated patients.

DISCUSSION

Our results demonstrate that tacrolimus is a significant independent predictor of survival in post-OLT HCV-infected patients. Many transplant physicians may find these results unexpected. In fact, we launched this investigation because we anticipated the opposite result. Our program preferentially administered cyclosporine to HCV-infected patients with the belief that this change in immunosuppression would lead to better outcomes based on *in vitro* data. After analyzing the results of our study, we reviewed the literature and found that the most definitive studies comparing the effect of CNIs on survival of HCV-infected patients had short follow-up (typically 1 year). Forman et al demonstrated a higher rate of graft loss in HCV-infected patients and showed the different outcomes were not apparent until after the first year of transplant (4). Berenguer et al published a meta-analysis comparing outcomes in HCV-infected liver transplant recipients and reported no difference in outcomes (25). All but one of the reviewed studies in this report evaluated 1-year outcomes. The single study reporting 5-year follow-up yielded results that mirror ours (18). This study was the long-term follow-up of the tacrolimus registration trial cohorts in which

Table 3. Hazard ratios of risk factors for patient survival and fibrosis progression

Risk factor	Patient survival		Advanced fibrosis	
	Univariate	Multivariable	Univariate	Multivariable
Cyclosporine vs. tacrolimus	1.37	1.45 (1.11–1.90)	NS	–
Donor age >60 years	1.49	NS	1.5*	NS
MELD $\geq$ 21	1.48	1.55 (1.14–2.11)	NS	–
MMF vs. azathioprine	0.57	NA [†]	NS	–
Steroids for chronic immunosuppression	1.48*	NS	NS	–
Recipient age >60 years	1.38*	NS	NS	–
Rejection >3 months	1.30*	NS	1.59	1.44 (1.05–1.98)
Gender	NS	–	NS	–
CIT >10 hours	NS	–	NS	–
Cytomegalovirus	NS	–	1.65	1.64 (1.21–2.21)
African American vs. Caucasian	NS	–	1.86	1.69 (1.11–2.59)
Hepatocellular carcinoma	NS	–	NS	–
Steroid-resistant rejection	NS	–	1.45*	NS

*Factors whose *P* value was between 0.1 and 0.05 and therefore were entered into the multivariable model.

[†]Not applicable; MMF was colinear with tacrolimus and therefore was not entered into the multivariable model.

MELD indicates Model for End-Stage Liver Disease; MMF, mycophenolate mofetil; CIT, cold ischemia time; NS, not significant.

Wiesner reported a significantly higher survival associated with tacrolimus (78.9%) compared with cyclosporine (60.5%) in HCV-infected patients.

Despite intriguing in vitro data that cyclosporine inhibits HCV viral replication, we must make post-OLT patient care decisions based on a drug's effect on patient survival and fibrosis progression (11–14). The survival difference was not explained by a difference in fibrosis progression. As expected from the fibrosis data, death due to recurrent disease was approximately the same in both groups. When evaluating the primary causes of death in the cyclosporine-treated group, rejection was higher in cyclosporine- than tacrolimus-treated

patients. This is in line with previous data showing that cyclosporine-treated patients have a higher incidence of rejection and with recent data showing an increased risk of malignancy (17, 18, 21, 22). We do not have accurate information on HCV treatment in this cohort and therefore cannot exclude that such treatment may have played a role in survival. However, if cyclosporine improved responsiveness to interferon therapy, we would have expected to see an improvement in survival or decreased fibrosis progression in these patients, which was not observed. Although the rejection rate was higher in the cyclosporine-treated patients, this does not explain the entire mortality difference. Since both HCV patients and non-HCV patients achieved a survival advantage with tacrolimus over cyclosporine, this may be a drug effect.

Of note, African Americans benefited the most from tacrolimus treatment, with an absolute 5-year

survival advantage of 20%. As African American recipients have historically had shorter post-OLT survival compared with Caucasians, it is very intriguing that CNi choice may mitigate this difference to some extent (23–25). However, the small number of African American recipients did not allow us to discern a reason for this difference. This observation requires confirmation in a larger multicenter cohort.

One limitation of the study was that many of the patients treated with tacrolimus were also treated with mycophenolate mofetil. Therefore, the individual effects of tacrolimus and mycophenolate mofetil could not be assessed.

The largest limitation of our study was its retrospective design, although the data were prospectively collected. Because of a programmatic protocol change in immunosuppression, fewer patients were treated with tacrolimus than cyclosporine before 2000, and after 2000 the opposite was true, with fewer patients treated with cyclosporine. This led to most patients being treated with tacrolimus in a different era than those treated with cyclosporine. Therefore, it is not possible to control for an “era” effect, as these two variables are colinear. Since the biggest difference between “eras” is likely immunosuppression and MELD at OLT (with the latter controlled for in multivariable modeling), we feel that our results remain valid despite this limitation.

In conclusion, tacrolimus affords HCV-infected post-OLT patients a long-term survival advantage over cyclosporine. CNi choice does not impact HCV fibrosis progression; however, tacrolimus does decrease the rate of rejection. Further study of CNi's impact on survival is needed in a larger multicenter cohort with long-term follow-up.

Table 4. Causes of death by calcineurin inhibitor (overall spread of death etiologies; *P* = 0.04)

Cause of death	Cyclosporine (N = 113)	Tacrolimus (N = 71)
Recurrent disease	27%	34%
Hepatocellular carcinoma/cholangiocarcinoma	11%	4%
Non–hepatocellular carcinoma malignancy	9%	6%
Infection	17%	14%
Cardiopulmonary	4%	13%
Surgical/primary graft nonfunction/biliary	7%	10%
Rejection	6%	0%
Neurological	4%	5%
Other	15%	14%

1. Gane E. The natural history and outcome of liver transplantation in hepatitis C virus-infected recipients. *Liver Transpl* 2003;9(11):S28–S34.
2. Melum E, Friman S, Bjoro K, Rasmussen A, Isoniemi H, Gjertsen H, Bäckman L, Oksanen A, Olausson M, Duraj FF, Ericzon BG. Hepatitis C impairs survival following liver transplantation irrespective of concomitant hepatocellular carcinoma. *J Hepatol* 2007;47(6):777–783.
3. Charlton M, Seaberg E, Wiesner R, Everhart J, Zetterman R, Lake J, Detre K, Hoofnagle J. Predictors of patient and graft survival following liver transplantation for hepatitis C. *Hepatology* 1998;28(3):823–830.
4. Forman LM, Lewis JD, Berlin JA, Feldman HI, Lucey MR. The association between hepatitis C infection and survival after orthotopic liver transplantation. *Gastroenterology* 2002;122(4):889–896.
5. Wiesner RH, Sorrell M, Villamil F. Report of the first International Liver Transplantation Society expert panel consensus conference on liver transplantation and hepatitis C. *Liver Transpl* 2003;9(11):S1–S9.
6. Thuluvath PJ, Krok KL, Segev DL, Yoo HY. Trends in post-liver transplant survival in patients with hepatitis C between 1991 and 2001 in the United States. *Liver Transpl* 2007;13(5):719–724.
7. Berenguer M, Palau A, Aguilera V, Rayón JM, Juan FS, Prieto M. Clinical benefits of antiviral therapy in patients with recurrent hepatitis C following liver transplantation. *Am J Transplant* 2008;8(3):679–687.
8. Samuel D, Bizollon T, Feray C, Roche B, Ahmed SN, Lemonnier C, Cohard M, Reynes M, Chevallier M, Ducerf C, Baulieux J, Geffner M, Albrecht JK, Bismuth H, Trepo C. Interferon-alpha 2b plus ribavirin in patients with chronic hepatitis C after liver transplantation: a randomized study. *Gastroenterology* 2003;124(3):642–650.
9. Garcia-Retortillo M, Forns X. Prevention and treatment of hepatitis C virus recurrence after liver transplantation. *J Hepatol* 2004;41(1):2–10.
10. Everson GT. Impact of immunosuppressive therapy on recurrence of hepatitis C. *Liver Transpl* 2002;8(10 Suppl 1):S19–S27.
11. Nakagawa M, Sakamoto N, Tanabe Y, Koyama T, Itsui Y, Takeda Y, Chen CH, Kakinuma S, Oooka S, Maekawa S, Enomoto N, Watanabe M. Suppression of hepatitis C virus replication by cyclosporin A is mediated by blockade of cyclophilins. *Gastroenterology* 2005;129(3):1031–1041.
12. Watashi K, Hijikata M, Hosaka M, Yamaji M, Shimotohno K. Cyclosporin A suppresses replication of hepatitis C virus genome in cultured hepatocytes. *Hepatology* 2003;38(5):1282–1288.
13. Watashi K, Metselaar HJ, van der Laan LJ. Interfering with interferon: re-igniting the debate on calcineurin inhibitor choice and antiviral therapy for hepatitis C virus recurrence. *Liver Transpl* 2008;14(3):265–267.
14. Henry SD, Metselaar HJ, Lonsdale RC, Kok A, Haagmans BL, Tilanus HW, van der Laan LJ. Mycophenolic acid inhibits hepatitis C virus replication and acts in synergy with cyclosporin A and interferon-alpha. *Gastroenterology* 2006;131(5):1452–1462.
15. Paeshuyse J, Kaul A, De Clercq E, Rosenwirth B, Dumont JM, Scalfaro P, Bartenschlager R, Neyts J. The non-immunosuppressive cyclosporin DEBIO-025 is a potent inhibitor of hepatitis C virus replication in vitro. *Hepatology* 2006;43(4):761–770.
16. Pan Q, Metselaar HJ, de Ruiter P, Kwekkeboom J, Tilanus HW, Janssen HL, van der Laan LJ. Calcineurin inhibitor tacrolimus does not interfere with the suppression of hepatitis C virus infection by interferon-alpha. *Liver Transpl* 2010;16(4):520–526.
17. U.S. Multicenter FK506 Liver Study Group. A comparison of tacrolimus (FK 506) and cyclosporine for immunosuppression in liver transplantation. *N Engl J Med* 1994;331(17):1110–1115.
18. Wiesner RH. A long-term comparison of tacrolimus (FK506) versus cyclosporine in liver transplantation: a report of the United States FK506 Study Group. *Transplantation* 1998;66(4):493–499.
19. O'Grady JG, Burroughs A, Hardy P, Elbourne D, Truesdale A; UK and Republic of Ireland Liver Transplant Study Group. Tacrolimus versus microemulsified cyclosporin in liver transplantation: the TMC randomised controlled trial. *Lancet* 2002;360(9340):1119–1125.
20. Fung JJ, Eliasziw M, Todo S, Jain A, Demetris AJ, McMichael JP, Starzl TE, Meier P, Donner A. The Pittsburgh randomized trial of tacrolimus compared to cyclosporine for hepatic transplantation. *J Am Coll Surg* 1996;183(2):117–125.
21. Berenguer M, Aguilera V, Prieto M, San Juan F, Rayón JM, Benlloch S, Berenguer J. Effect of calcineurin inhibitors on survival and histologic disease severity in HCV-infected liver transplant recipients. *Liver Transpl* 2006;12(5):762–767.
22. Lucey MR, Abdelmalek MF, Gagliardi R, Granger D, Holt C, Kam I, Klintmalm G, Langnas A, Shetty K, Tzakis A, Woodle ES. A comparison of tacrolimus and cyclosporine in liver transplantation: effects on renal function and cardiovascular risk status. *Am J Transplant* 2005;5(5):1111–1119.
23. Samonakis DN, Triantos CK, Thalheimer U, Quaglia A, Leandro G, Teixeira R, Papatheodoridis GV, Sabin CA, Rolando N, Davies S, Dhillon AP, Griffiths P, Emery V, Patch DW, Davidson BR, Rolles K, Burroughs AK. Immunosuppression and donor age with respect to severity of HCV recurrence after liver transplantation. *Liver Transpl* 2005;11(4):386–395.
24. Merion RM, Schaubel DE, Dykstra DM, Freeman RB, Port FK, Wolfe RA. The survival benefit of liver transplantation. *Am J Transplant* 2005;5(2):307–313.
25. Berenguer M, Royuela A, Zamora J. Immunosuppression with calcineurin inhibitors with respect to the outcome of HCV recurrence after liver transplantation: results of a meta-analysis. *Liver Transpl* 2007;13(1):21–29.