Received: 2015.07.13
Accepted: 2015.08.05
Published: 2015.11.26

Long-Term Renal Function in Liver Transplant Recipients After Conversion From Calcineurin Inhibitors to mTOR Inhibitors

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Source of support: Part of this work was sponsored by Novartis

Background: Renal dysfunction often occurs in liver transplant (LT) recipients receiving calcineurin inhibitor (CNI)-based immunosuppressive regimens, increasing morbidity and mortality rates. Replacement of CNIs by mTOR inhibitor-based immunosuppressive protocols may prevent renal impairment in LT recipients.

Material/Methods: Outcomes in patients who underwent LT between 1996 and 2010 at our center and who were switched from CNI-based to mTOR inhibitor-based immunosuppression were retrospectively analyzed. Renal course, hyperlipidemia, and graft rejection were assessed in patients maintained on this CNI-free regimen for at least 24 months.

Results: Of the 85 patients switched from CNI-based to mTOR inhibitor-based, CNI-free immunosuppression, 78 met the inclusion criteria. Within the first 6 weeks after switching, the covariable adjusted estimated glomerular filtration rate (eGFR) increased 5.6 mL/min [95% confidence interval 2.6–8.7 mL/min, $p < 0.001$], but there were no further statistically noticeable changes in eGFR. Concentrations of cholesterol and triglycerides increased statistically, noticeable within the first 12 months after drug conversion. Histologically proven graft rejection was observed in 4 patients (5.1%) after conversion.

Conclusions: Conversion from CNI-based to CNI-free, mTOR inhibitor-based immunosuppression after LT is safe and can result in significant renal recovery. CNI-free, mTOR inhibitor-based immunosuppression is a potential option for patients with contraindications for CNIs and for LT recipients with rapid reduction in kidney function due to CNIs.

MeSH Keywords: Calcineurin • Immunosuppression • Liver Transplantation • Renal Insufficiency • TOR Serine-Threonine Kinases

Full-text PDF: <http://www.annalsoftransplantation.com/abstract/index/idArt/895320>

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Background

Technical and pharmacologic developments have resulted in longer-term survival after liver transplantation (LT) [1]. Most long-term complications after LT, including renal failure, are caused by the adverse effects of immunosuppressant treatments [2]. Current treatment guidelines emphasize the importance of individualized immunosuppression regimens that both avoid the adverse effects of immunosuppressive agents and reduce the risk of acute rejection [2]. Calcineurin inhibitors (CNIs) have been a cornerstone of immunosuppressive therapy after solid organ transplantation, significantly reducing the risk of graft rejection. CNIs, however, are associated with severe adverse effects, such as renal dysfunction, resulting in the reduction of their short- and long-term use [3,4]. Furthermore, mortality rates are higher in LT recipients with vs. without post-transplant renal dysfunction [5–7]. Therefore, current immunosuppressive strategies after LT are designed to avoid nephrotoxic drug regimens, favoring reductions in CNIs and CNI-free protocols [8]. Some of these protocols include switching from CNI-based immunosuppression to immunosuppressive regimens based on inhibitors of mechanistic target of rapamycin (mTOR) or combinations of mTOR inhibitors with reduced doses of CNIs. Although these strategies can lead to reduced nephrotoxicity and improved renal function [9,10], CNI-free immunosuppressive regimens have been associated with a higher risk for acute graft rejection [11,12]. This study was designed to evaluate the course of renal function during the first 24 months after conversion from a CNI (tacrolimus or cyclosporine) to an mTOR inhibitor (sirolimus or everolimus). In addition, the rates of dyslipidemia and graft rejection in these LT recipients were determined.

Material and Methods

This retrospective study enrolled patients who underwent LT between 1996 and 2010 at the University Hospital of Muenster. After LT, all patients were first started on an immunosuppressive protocol consisting of CNI-based immunosuppression. Patients who were subsequently converted from a CNI-based immunosuppressive regimen to a CNI-free, mTOR inhibitor-based regimen and were maintained on this regimen for at least 24 months were included in this study.

The study was conducted according to the ethics principles of the Declaration of Helsinki and was approved by the institutional review board of the University Hospital of Muenster.

Patient data, including age; sex; and creatinine, total blood cholesterol, and triglyceride concentrations, were collected at the time of drug conversion and at defined time points during follow-up. All changes in immunosuppressive drugs were documented.

Data on the clinical course of patients were collected from patient files for at least 24 months after drug conversion.

All included patients received mTOR inhibitor-based immunosuppression for at least 24 months after conversion. Patients who also received CNI were not included. We also excluded patients who switched from mTOR inhibitor-based immunosuppression to another immunosuppressive regimen or who discontinued mTOR inhibitors; those who experienced loss of graft function; and patients who died or were lost to follow-up.

Renal function was evaluated by determining the estimated glomerular filtration rate (eGFR) using the MDRD4 formula [13].

Immunosuppressive regimen

The standard post-LT immunosuppressive regimen at our center included tacrolimus or cyclosporine with/without mycophenolate mofetil and with/without prednisolone. The CNI-free regimen included everolimus or rapamycin with/without mycophenolate mofetil and with/without prednisolone.

Statistical analysis

Statistical analyses were performed using SAS software, version 9.4, for Windows (SAS Institute, Cary, NC, USA). Inferential statistics were intended to be exploratory rather than confirmatory. P-values were used to generate new hypotheses and represent only a metric measure of evidence against the respective null hypothesis. Thus, neither a global significance level nor local levels were determined, and there were no adjustments for multiplicity. P-values ≤ 0.05 were considered statistically significant.

The primary target parameter was eGFR (mL/min). Secondary parameters included cholesterol (mmol/L) and triglyceride (mmol/L) concentrations. Standard univariate statistical analyses were performed to describe demographic and clinical parameters. Categorical variables are reported as absolute and relative frequencies. Normally distributed continuous variables are reported as mean $\pm$ standard deviation, and non-normally distributed continuous variables as median (minimum, maximum).

Longitudinal analyses were performed using multivariable linear mixed models (LMM) to quantify differences between the 2 immunosuppressive regimens. Target variables were eGFR and cholesterol and triglyceride concentrations. Due to the skewed distribution of triglyceride concentrations, they were log-transformed. Details of the model are described in the legends to Tables 1 and 2. Pairwise comparisons between the regimens at each time point were estimated within the multivariable LMM. Results are reported as regression coefficients and corresponding 95% confidence intervals (95% CI).

Table 1. Factors associated with renal function (eGFR, ml/min), as determined by a linear mixed model.

Independent variable	Model-based estimates of eGFR (ml/min)			P-value
	Estimate	Lower 95% confidence limit	Upper 95% confidence limit	
Intercept	−2972.7	−7228.2	1282.8	0.1679
Sex (m vs. f)	10.9	0.6	21.2	0.0380
Time points				0.0094
6 weeks vs. baseline	5.6	2.6	8.7	0.0003
3 months vs. baseline	5.5	2.6	8.3	0.0002
6 months vs. baseline	4.4	0.9	7.8	0.0142
12 months vs. baseline	5.2	1.8	8.7	0.0033
18 months vs. baseline	4.6	1.3	7.9	0.0061
24 months vs. baseline	4.3	0.7	7.8	0.0182
Year of LT (per year: 1996 to 2010)	1.52	−0.6	3.6	0.1515
Age in years	−0.6	−1.0	−0.3	<0.0001
Time from LT to drug conversion (months)	0.02	−0.2	0.2	0.8385
Diabetes (yes vs. no)	−3.3	−11.8	5.3	0.4510
Hypertension (yes vs. no)	−7.0	16.4	2.4	0.1450
HCV (yes vs. no)	8.9	−12.6	30.3	0.4176
Drug after conversion (sirolimus vs. everolimus)	5.5	−2.9	13.9	0.1991
Drug before conversion (cyclosporine vs. tacrolimus)	3.5	−18.7	25.7	0.7576

Regression coefficient estimates and 95% confidence limits of fixed effects on eGFR (ml/min). Covariates were: year of LTx (year: range 1996 to 2010), gender, age at LTx, time since LTx to drug conversion, CNI inhibitor before conversion, diabetes, hypertension, HCV. A random intercept for each patient and an unstructured covariance structure (UN) for the residuals were included. The variances were estimated using the empirical sandwich estimator. P-values are from the related F-tests or t-tests. eGFR – estimated glomerular filtration rate; HCV – hepatitis C virus.

Results

During the period from 1996 to 2010, 85 patients were switched from CNI-based immunosuppression to mTOR-based, CNI-free immunosuppression. Of these, 4 (4.7%) discontinued treatment because of adverse events (2 each due to proteinuria and pancytopenia), and 3 (3.5%) were lost to follow-up. The remaining 78 patients (24 females, 54 males; mean age at transplantation, 53.1±12.9 years) met the inclusion criteria for this study and were included in the statistical analysis. Median time from LT to drug conversion was 12 months (range, 1–180 months). Table 3 shows the demographic and clinical characteristics of the study population. Of the 78 included patients, 39 (50%) were switched from tacrolimus to sirolimus, 32 (41%) from tacrolimus to everolimus, 3 (4%) from cyclosporine to sirolimus, and 4 (5%) from cyclosporine to everolimus.

Because there were no differences among the immunosuppressive regimens in the time courses of all target parameters, an interaction effect between the regimens and time points was not included in the LMMs.

Course of renal function

Baseline eGFR at the time of drug conversion was 55.2±25.3 mL/min. Six weeks after drug conversion, eGFR showed a covariable adjusted increase of 5.6 mL/min [95% CI 2.6–8.7 mL/min, $p<0.001$]. Table 1 shows the course of eGFR as calculated by the multivariate LMM. Changes from baseline in eGFR at all time points were statistically significant, but changes from week 6 were not. Furthermore, multivariate analysis showed that only female sex ($p=0.038$) and increased age ($p<0.001$) negatively influenced eGFR. Increased eGFR after conversion was independent of immunosuppressive regimens before and after

Table 2. Factors associated with total cholesterol concentrations, as determined by a linear mixed model.

Independent variable	Model-based estimates of Cholesterol (mmol/L)			P-value
	Estimate	Lower 95% confidence limit	Upper 95% confidence limit	
Intercept	3231.4	−5394.9	11858	0.4573
Sex (m vs. f)	−22.9	−48.8	3.0	0.0828
Point of time				<0.0001
12 months vs. baseline	29.6	18.6	40.7	<0.0001
18 months vs. baseline	27.6	16.5	38.6	<0.0001
24 months vs. baseline	17.3	5.3	29.3	0.0049
Year of LTx (per year: 1996 to 2010)	−1.5	−5.8	2.7	0.4835
Age in years	0.02	−0.8	0.9	0.9707
Time from LT to drug conversion (months)	0.2	−0.6	0.1	0.1817
Diabetes (yes vs. no)	4.1	−19.0	27.3	0.7271
Hypertension (yes vs. no)	1.3	−17.6	20.2	0.8960
HCV (yes vs. no)	−13.2	−42.2	15.7	0.3682
Drug after conversion (sirolimus vs. everolimus)	9.6	−9.0	28.2	0.3095
Drug before conversion (cyclosporine vs. tacrolimus)	18.8	−21.0	58.9	0.3520

Regression coefficient estimates and 95% confidence limits of fixed effects on cholesterol (mmol/L). Covariates were: year of LTx (year: range 1996 to 2010), gender, age at LTx, time since LTx to drug conversion, CNi inhibitor before conversion, diabetes, hypertension, HCV. A random intercept for each patient and an unstructured covariance structure (UN) for the residuals were included. The variances were estimated using the empirical sandwich estimator. P-values are from the related F-tests or t-tests. Abbreviations: HCV, hepatitis C virus.

conversion; time between LT and conversion; year of LT; and presence of diabetes, hypertension, and HCV infection. Table 4 shows descriptive statistics of eGFR during the observation time.

Lipid changes after drug conversion

During the first 12 months after conversion, mean blood cholesterol concentrations increased from 187.1 ± 46.9 mmol/L to 216.7 ± 57.7 mmol/L. Adjusted blood cholesterol concentration increased by 29.6 mmol/L [95% CI 18.6–40.7 mmol/L, $p < 0.001$]. No further increases were observed after 12 months. Table 2 shows changes in cholesterol relative to different variables. Except for time from conversion, no other variable had a statistically significant effect on cholesterol concentration.

Mean serum triglyceride levels increased from 168.9 ± 84.6 mmol/L at baseline to 210.3 ± 135 mmol/L at 12 months, but decreased to 179.0 mmol/L at 24 months. Triglyceride concentration increased significantly over the first 12 months ($p = 0.0034$), but the change from baseline to 24 months was not significant ($p = 0.3668$).

Of the 78 patients, 25 (32%) required treatment with statins due to hyperlipidemia that developed after conversion to mTOR-inhibiting agents.

Rejection

During the 24 months after conversion to the mTOR inhibitor-based immunosuppression, 4 patients (5.1%) experienced histologically acute graft rejection.

In the multivariate adjusted model, eGFR and lipid concentrations did not differ noticeably in patients switched to everolimus and sirolimus. Because of the small number of patients who experienced graft rejection, the difference in rejection rate between patients switched to everolimus and sirolimus could not be determined.

Table 3. Demographic and clinical characteristics of the LT recipients included in this study.

Age at LT (years)	53.1±12.9/55 (12, 71)	
Age at drug conversion (years)	55.8±12.5/57 (19, 76)	
Sex (females/males)	24 (30.8%)/54 (69.2%)	
Time from LT to drug conversion (months)	31.0±40.6/12 (1, 180)	
Patients with HCV	7	(9.0%)
Arterial Hypertension	48	(61.5%)
Diabetes	31	(39.7%)
IS medication before conversion		
Tacrolimus	71	(91.0%)
Cyclosporine	7	(9.0%)
IS medication after conversion		
Sirolimus	42	(53.8%)
Everolimus	36	(46.2%)
Indications for LT		
Hepatocellular carcinoma	22	(28.2%)
Alcohol-induced cirrhosis	15	(19.2%)
Hepatitis B/C	11	(14.1%)
Primary sclerosing cholangitis	7	(9.0%)
Cryptogenic liver cirrhosis	5	(6.4%)
Polycystic liver disease	5	(6.4%)
Autoimmune hepatitis	4	(5.1%)
Biliary atresia	2	(2.6%)
AAT deficiency	2	(2.6%)
Primary biliary cirrhosis	1	(1.3%)
Idiopathic acute liver failure	1	(1.3%)
Familial amyloid polyneuropathy	1	(1.3%)
Wilson's disease	1	(1.3%)
Secondary sclerosing cholangitis	1	(1.3%)

Results are reported as mean ± standard deviation; median (minimum, maximum); or absolute and relative frequencies. AAT – alpha 1 antitrypsin; HCV – hepatitis C virus; LT – liver transplantation; IS – immunosuppression.

Discussion

Clinical studies have shown that both reduction of CNI dose and CNI discontinuation were associated with significant renal recovery [15,16]. The findings shown here are consistent with these earlier results, in that elimination of CNIs by conversion to mTOR inhibitor-based immunosuppression resulted in a significant improvement in renal function, as shown by the increase in eGFR. Interestingly, this improvement occurred within 6 weeks after drug conversion, with no further improvement observed thereafter.

Renal recovery after conversion was independent of the time period between LT and conversion, indicating that renal recovery can occur after CNI elimination despite existing CNI-related parenchymal renal damage [17,18]. A multivariate adjusted model found that the only factors having a noticeable negative influence on renal function (e.g., lower eGFR) were increased age and female sex, consistent with studies showing that female sex and increased age were associated with increased renal vulnerability to noxious agents and to drug toxicity [19–21].

Table 4. Descriptive statistics of GFR over time.

Time point	GFR (mL/min)							
	N	Mean	Standard Deviation	Minimum	Q25	Median	Q75	Maximum
Conversion	78	55.2	25.3	11.0	35.0	50.0	75.0	137.3
6 weeks	78	60.9	25.5	11.9	40.8	57.0	78.1	128.3
3 months	78	60.7	24.8	8.0	43.0	56.0	77.9	134.8
6 months	78	59.6	23.1	16.0	43.0	55.0	76.4	116.5
12 months	78	60.5	25.3	16.0	42.0	55.5	76.1	150.7
18 months	78	59.8	25.0	16.0	43.0	53.7	70.2	150.7
24 months	78	59.5	24.7	15.8	43.0	55.0	75.3	136.2

Q25 – 25% quantile; Q75 – 75% quantile.

Conversion to mTOR-inhibitor-based immunosuppression was also associated with the development of mixed hyperlipidemia. Total cholesterol and triglyceride concentrations increased significantly during the first 12 months after conversion. In all of these patients, hyperlipidemia was managed by dietary measures or by administration of lipid-lowering agents.

Although the rejection rate was found to be significantly greater in LT recipients after CNI withdrawal [12], our study found that the rejection rate after switching to mTOR-inhibiting agents was 5.1%, similar to that in patients maintained on CNIs [11,12]. This discrepancy may have been due to the timing of drug conversion, in that our patients were converted a mean of 31 months after LT, whereas in the earlier study CNIs were withdrawn a mean of 30 days after LT [12]. All rejections in our study were successfully managed using pulse steroid therapy and adjustment of blood trough levels of immunosuppressive agents.

To the best of our knowledge, no previous study has compared the efficacy and safety of everolimus and sirolimus after LT. Our study found that these 2 drugs were not different in inducing renal improvement and in their incidence of hyperlipidemia.

In conclusion, conversion from CNIs to mTOR-inhibiting agents can result in improved renal function in LT recipients. Moreover, renal recovery can be achieved even when patients are switched many years after LT. These results also show that mTOR inhibitors are safe in LT recipients and are associated with a reasonable allograft rejection rate in stable LT recipients. Most of the adverse effects could be managed without drug withdrawal.

Conclusions

In 2013, the US FDA approved everolimus as the first mTOR-inhibiting agent for the prophylaxis of allograft rejection after LT. Due to the higher rejection rate after withdrawal [12], the FDA approved everolimus in combination with reduced tacrolimus. Our findings, however, indicate that withdrawal from CNIs and treatment with mTOR-inhibitor-based immunosuppression may be an option in LT patients with serious CNI-associated adverse effects and rapid loss of renal function.

Conflict of interest

Part of this work was sponsored by Novartis.

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