Abstract

Objectives: This study sought to assess the long-term efficacy and safety of conversion from a calcineurin inhibitor-based immunosuppressive regimen to sirolimus monotherapy in liver transplant recipients with renal dysfunction.

Materials and Methods: Twenty-five liver transplant recipients with calcineurin inhibitor-based immunosuppression were included in this single-center, prospective study. Indications were renal dysfunction, avoidance of tumor recurrence, combination renal dysfunction and avoidance of tumor recurrence, and calcineurin inhibitor-related adverse effects.

Results: Mean interval between liver transplant and initiation of sirolimus monotherapy was 51.7 months. The mean follow-up was 75.6 months. The mean ± SD sirolimus whole-blood trough level was 9.0 ± 2.8 ng/mL after 6 months and 6.0 ± 1.8 ng/mL after 18 months. No rejection episode occurred. There was an improvement of the mean creatinine level: 156.1 ± 54.9 µmol/L before conversion versus 129.1 ± 34.7 µmol/L approximately 3 years after conversion (P < .05). The glomerular filtration rate, measured by technetium Tc-99m-diethylenetriamine penta acetic aerosol scintigraphy, improved from 27.4 ± 6.8 mL/min/1.73 m² before conversion to 43.3 ± 6.3 mL/min/1.73 m² at final follow-up. Proteinuria increased after conversion to sirolimus after 6 months (P < .05) and at last follow-up. The systolic blood pressure decreased from 151.5 ± 20.2 to 132.1 ± 19.4 mm Hg, and the diastolic from 89.7 ± 11.2 to 82.1 ± 9.1 mm Hg at last follow-up. Serum cholesterol and serum triglyceride levels were nearly unchanged. However, 50% of the patients were treated with lipid-lowering agents. Four patients had sirolimus-induced adverse effects (thrombocytopenia, gingival hyperplasia, oral ulceration).

Conclusions: Conversion from calcineurin inhibitors to sirolimus monotherapy after liver transplant results in stabilization of renal function in 75% to 85% of cases and of blood pressure, without increased risk of rejection. The spectrum of adverse effects is low.

Key words: Calcineurin inhibitor, Nephrotoxicity

Introduction

Overall survival after liver transplant has improved during the last several years, with most patients living well beyond 5 years.1 The calcineurin inhibitors (CNIs) cyclosporine and tacrolimus, mycophenolate mofetil, and induction therapies have drastically reduced the risk of early and late graft loss. There is increasing emphasis on the management of complications associated with long-term immunosuppression. Although the cause of renal dysfunction after liver transplant is likely to be multifactorial, there is evidence that the prolonged use of both tacrolimus and cyclosporine contributes to renal insufficiency and to end-stage renal disease.2-4 Thirteen years after transplant, severe renal dysfunction occurred in 18.1% of liver transplant recipients receiving CNI-based immunosuppression, and 9.5% needed hemodialysis.5 Long-term survival of patients with end-stage renal disease requiring hemodialysis therapy is low, with a reported survival rate of only 27% at 6 years.1
Arterial hypertension, hyperlipidemia, and hyperuricemia are further nonimmunologic adverse effects of CNIs. In addition, based on experimental and clinical studies, CNIs enhance cancer cell growth.6-10 Sirolimus inhibits interleukin-2–mediated signal transduction pathway through binding to a specific cell cycle regulator protein called mammalian target of rapamycin (mTOR). In recent nonrandomized trials, investigators have shown that replacement of CNIs by sirolimus in patients receiving liver, kidney, and heart transplants can improve renal function in selected patients.11-14 Other benefits of sirolimus are favorable effects on posttransplant hypertension and antitumor activity.15, 16 On the other hand, relevant adverse effects of sirolimus in liver transplant recipients have been reported by Montalbano and associates.17 Despite theoretical advantages of sirolimus compared with CNIs, more validated clinical long-term data are required to define the role of sirolimus in the immunosuppression strategy after liver transplant. Recent data suggest that the reported advantage of sirolimus-based immunosuppression on long-term kidney function may be overstated.18

The aim of this prospective single-center study was to assess the long-term efficacy and safety of conversion from a CNI-based immunosuppressive regimen to sirolimus monotherapy in liver-transplant recipients with renal dysfunction, CNI-associated complications, or a history of malignant neoplasm.

Materials and Methods

Patients and inclusion criteria

Patient characteristics are summarized in Table 1. From March 2002 to February 2004, 25 patients were included who underwent liver transplant at least 6 months previously and had a stable graft function. Four patients had received a second liver graft. Patients with severe rejection episodes (ie, steroid-resistant or repeated), other renal diseases, and severe hyperlipidemia (ie, cholesterol level > 6 mmol/L) were excluded. Stable graft function was defined as stable aspartate aminotransferase and alanine aminotransferase concentrations (< 1.5-fold the upper limit) and normal bilirubin level.

Indications for conversion to sirolimus monotherapy were renal dysfunction (n=16), a combination renal dysfunction and avoidance of tumor recurrence (n=3), and CNI-related adverse effects (n=2), are shown in Table 2. Renal dysfunction was defined as serum creatinine level of 120 μmol/L or higher (reference range, 45 to 85 μmol/L), measured on at least 2 successive occasions more than 3 months apart. The cause of renal dysfunction was presumed to be the result of CNI use because kidney biopsies were not routinely performed as part of the study. Informed consent was obtained from all patients. All protocols were approved by the local ethics committee of the institution before the study began, and they conformed to the ethical guidelines of the 1975 Helsinki Declaration.

Conversion protocol

Sirolimus therapy was introduced in stages. It was started at a dosage of 1 mg/d in the patients who had not previously received sirolimus. In all patients, with or without sirolimus treatment, dosing was guided by trough levels to achieve therapeutic blood levels of 6 to 9 ng/mL. From the time of obtaining target sirolimus levels, the CNI dosage was reduced by 25% of the initial dose per week. One patient had been receiving azathioprine, and this treatment regimen was stopped on initiation of sirolimus therapy (patient 16, Table 2). In patients who were treated with CNI and mycophenolate mofetil, mycophenolate mofetil dosing was stopped after withdrawal of CNI therapy and obtaining stable trough levels of sirolimus. Four weeks after discontinuation of treatment with CNIs and mycophenolate mofetil, the corticosteroid dose was reduced stepwise every week and withdrawn if possible. Six months after sirolimus monotherapy, the whole-blood target levels were adjusted to approximately 6 ng/mL.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>n</th>
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<tbody>
<tr>
<td>Sex (male/female)</td>
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<tr>
<td>Age (mean years ± standard deviation)</td>
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<tr>
<td>Cause of liver disease</td>
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<tr>
<td>Alcoholic/cryptogenic cirrhosis</td>
<td>14</td>
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<tr>
<td>Primary biliary cirrhosis</td>
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<tr>
<td>Primary sclerosing cholangitis</td>
<td>1</td>
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<tr>
<td>Cirrhosis caused by hepatitis B</td>
<td>1</td>
</tr>
<tr>
<td>Morbus Wilson disease</td>
<td>2</td>
</tr>
<tr>
<td>Budd-Chiari syndrome</td>
<td>1</td>
</tr>
<tr>
<td>Malignant neoplasm</td>
<td>5*</td>
</tr>
<tr>
<td>First/second transplant</td>
<td>21/4</td>
</tr>
<tr>
<td>Rejection (mild; corticosteroid treatment)</td>
<td>3</td>
</tr>
<tr>
<td>Median months from transplant to conversion</td>
<td>25.7 (6-45)</td>
</tr>
</tbody>
</table>

*Three cases of hepatocellular carcinoma, 1 case of hilar cholangiocarcinoma, and 1 case of liver metastases of neuroendocrine carcinoma.

Table 1. Patient characteristics.
Outcome parameters
Renal function was monitored by measurement of serum creatinine levels and the glomerular filtration rate, assessed by technetium Tc-99m diethylenetriamine pentaacetic aerosol (DTPA) scintigraphy. Renal function, blood pressure, fasting cholesterol and triglyceride levels, blood cell count, serum uric acid level, blood glucose level, and proteinuria were assessed before conversion of immunosuppression, every 3 months during the first year after the beginning of conversion, every 6 months during the second year after conversion, and yearly afterward. Transaminases (aspartate and alanine), γ-glutamyl-transpeptidase, alkaline phosphatase, and bilirubin levels were assessed during the period of conversion every 3 weeks and afterward at the same time points as the other already mentioned parameters. All medication and drug-related adverse effects were documented.

Statistical Analyses
Parametric data were compared using paired Wilcoxon rank sum test. Parametric data were expressed as mean ± SD. A P value less than .05 was regarded as significant. Statistical analyses were performed with SPSS software (SPSS: An IBM Company, version 10.0, IBM Corporation, Armonk, New York, USA).

Results
Baseline data
Demographic data are shown in Table 1. Twenty-five patients satisfied inclusion and exclusion criteria. Among these 25 patients (mean age, 50.1 ± 12.3 y; range, 30 to 63 y, 6 women, 19 men), 20 were switched to sirolimus monotherapy because of renal dysfunction alone or in combination with avoidance of tumor recurrence (4 patients). Among these 20 patients, 18 patients had moderate renal insufficiency (serum creatinine level, 128 to 167 µmol/L), and 2 patients had severe renal insufficiency (serum creatinine, 218 to 227 µmol/L) (Table 2). Further indications for conversion to sirolimus monotherapy were avoidance of tumor recurrence (n=3) and CNI-related adverse effects (n=2). Sirolimus monotherapy was started a mean of 51.7 months (range, 6 to 102 mo) after transplant. Reasons for transplant are given in Table 1.
At time of conversion, mean values of liver function were within the reference range except for a slight increase of γ-glutamyl transpeptidase and alkaline phosphatase. In detail, the results were as follows: Quick time, 97.6% ± 9.2%; albumin, 43.6 ± 8.5 g/L; bilirubin, 10.9 ± 5.8 μmol/L; aspartate aminotransferase, 0.44 ± 0.2 μkat/L; alanine aminotransferase, 0.44 ± 0.2 μkat/L; γ-glutamyltranspeptidase, 1.27 ± 0.6 μkat/L; and alkaline phosphatase, 3.7 ± 1.5 μkat/L.

Mean follow-up of patients receiving sirolimus monotherapy was 75.6 months (range, 64 to 86 mo). One patient died of neuroendocrine carcinoma 30 months after the start of sirolimus monotherapy, and another patient died of cardiac infarction 21 months after initiation of sirolimus monotherapy, both with functioning grafts. The latter patient had hypertension and hypercholesterinemia as risk factors for cardiac events. In the remaining 23 patients, no grafts were lost. No rejections occurred after the start of monotherapy. The immunosuppressive protocols before sirolimus monotherapy and the individual reasons for monotherapy are provided in Table 2. Immunosuppression protocol before conversion was cyclosporine-based in 7 patients and tacrolimus-based in 18 patients (Table 2). The mean sirolimus whole-blood trough level was 9.1 ± 2.8 ng/mL after 6 months and 6.3 ± 1.8 ng/mL after 18 months.

Renal function

In the 20 patients with renal dysfunction, the glomerular filtration rate (GFR) significantly increased from 27.4 ± 6.8 mL/min/1.73 m² before conversion to 39.3 ± 10.1 mL/min/1.73 m² at 12 months after the beginning of sirolimus monotherapy (P < .05). In the period from 12 months to 60 months after conversion, the technetium Tc-99m DTPA values remained stable (Figure 1).

Mean serum creatinine level showed a significant decrease from 156.1 ± 54.9 μmol/L before conversion to 129.1 μmol/L 12 months afterward (P < .05), with stable values at each later measurement point (Figure 2). Serum creatinine level improved in 17 of 20 patients with renal dysfunction. In 2 patients, included in this study because of nephrotoxicity, serum creatinine levels before conversion were 218 and 227 mol/L. In both patients, only slight recovery of kidney function could be found.

The number of patients with urinary protein excretion more than 1 g/d increased from 2 patients before conversion to 8 patients at last follow-up. During the study, there was no change in the use of drugs that could affect the degree of proteinuria. Proteinuria increased after conversion to sirolimus from 225 ± 23.2 mg/d at initiation of monotherapy to 372 ± 31.5 mg/d after 6 months (P < .05) and to 432 ± 35.2 mg/d at final follow-up. However, no statistically significant change in GFR was observed in patients with new-onset proteinuria during the follow-up.

Blood pressure, blood values, and blood lipids

Twelve of our patients had hypertension. After conversion to sirolimus monotherapy, a stabilization of blood pressure was found. The systolic blood pressure decreased from 151.5 ± 20.2 mm Hg to 132.1 ± 19.4 mm Hg, and the diastolic blood pressure decreased from 89.7 ± 11.2 mm Hg to 82.1 ± 9.1 mm Hg.
Hg at the last follow-up (Figure 3). After conversion to sirolimus monotherapy, the number of antihypertensive medications could be reduced in 4 patients (from 2 to 1 medication) and omitted in 3 patients.

Overall, clinical tolerance of sirolimus monotherapy was very good. Hemoglobin levels (before conversion, 8.2 ± 1.6 mmol/L; last follow-up, 8.1 ± 1.4 mmol/L), leukocyte counts (before conversion, 6.1 ± 2.4 × 10⁹/L; last follow-up, 5.3 ± 1.8 × 10⁹/L), and platelet counts (before conversion, 195 ± 74 × 10⁹/L; last follow-up, 212 ± 77 × 10⁹/L) did not change significantly (Figures 4 and 5). Furthermore, total cholesterol concentrations (before conversion, 6.02 ± 1.28 mmol/L; last follow-up, 5.18 ± 0.93 mmol/L) and triglyceride concentrations (before conversion, 1.9 ± 0.62 mmol/L; last follow-up, 2.2 ± 1.1 mmol/L) remained stable (Figures 6 and 7). However, 11 patients received lipid-lowering medication before conversion, and 12 received such medication after conversion.

**Adverse effects**
After conversion, 4 patients experienced slight adverse effects (thrombocytopenia, gingival hyperplasia, oral ulceration). Only 1 patient had to have sirolimus therapy withdrawn because of acneiform rash, and therapy was switched to everolimus.

**Tumor recurrence**
Among the 7 patients with conversion to sirolimus monotherapy because of a history of cancer, 1 patient died of liver metastases of a neuroendocrine small-bowel cancer 7.5 years after transplant and 2.5 years after the beginning of sirolimus monotherapy.
Discussion

Current immunosuppressive strategies focus on individually tailored protocols, CNI-sparing or avoiding regimens, and corticosteroid-avoiding protocols. These concepts aim to minimize drug adverse effects with few rejection episodes, to reduce long-term drug-related morbidity and mortality, including risk of cancer, and to achieve “almost tolerance” in the future. One of these innovative immunosuppressive regimens is single-drug maintenance immunosuppression. Our study is, to our knowledge, one of the first published long-term studies demonstrating feasibility and safety of sirolimus monotherapy introduced after liver transplant in a selected group of patients.

Renal dysfunction is among the most problematic complications seen after liver transplant. Liver transplant recipients as a group experience a substantial decline in the GFR after liver transplant, and end-stage renal disease will develop in up to 10% of liver transplant recipients by 10 years after transplant.19, 20 Many factors, including increased bile acids, endotoxins, circulating immune complexes and various cytokines, nephrotoxic drugs, and hitherto unrecognized factors, predispose patients with cirrhosis to renal dysfunction.21-23 Some of these factors also may play a role in progression of renal disease after liver transplant.21, 24, 25 Previous studies have attempted to identify risk factors for posttransplant renal failure. Nair and associates13 found posttransplant alcohol use and diabetes mellitus were independent predictors of late-onset renal failure, especially in patients with hepatitis C virus. Pawarode and associates26 identified risk factors that independently predicted the need for immediate dialysis and the development of permanent renal dysfunction and severe renal failure after liver transplant. A documented history of renal insufficiency (creatinine level > 1.2 mg/dL) or a baseline GFR below 7 mL/min/1.73 m² predicted the development of permanent renal dysfunction as we have defined it. Diabetes, coronary artery disease, and primary graft nonfunction predicted the development of chronic renal failure.26

However, CNIs have a substantial role by causing both structural and functional changes in the kidneys. In the acute phase of toxicity, there is a reduction in GFR accompanied by a decrease in renal blood flow mediated primarily by selective constriction of the afferent renal arteriole through upregulation of angiotensin II receptors and augmentation of angiotensin II-induced calcium responses.27 With continued exposure to CNI, structural changes occur in a dose-dependent manner and often are progressive and irreversible.28 It is important to note that early in the course of therapy with CNIs, neither serum creatinine level nor GFR may accurately reflect the degree of renal damage because of compensatory hyperfiltration of the remaining glomeruli.29 Over time, this adaptive response, which initially may falsely normalize serum creatinine level and GFR, can be detrimental and contribute to renal failure.30 Most of our patients had been receiving CNI therapy for years before conversion to sirolimus therapy. Although 17 of 20 patients had improvement in both serum creatinine level and GFR, improvement was not uniform, and moreover, not all patients had complete normalization. Two patients with severe CNI toxicity did not benefit from conversion regarding kidney function.

There are several explanations for this observation. The cause of renal dysfunction in liver transplant recipients is multifactorial. Based on histopathologic studies, it is assumable that the patients have varying degrees of chronic CNI-induced nephrotoxicity, including tubular toxicity, vascular-interstitial structural lesions, and interstitial fibrosis.31 Falkenhain and associates32 found that patients with heart and liver transplant had a progressive increase in renal arteriolar hyalinosis and in the percentage of renal glomeruli demonstrating global sclerosis with increasing time after transplant.
In addition, there was a statistically significant relation between arteriolar hyalinosis and global glomerulosclerosis. In addition to renal toxicity owing to CNI, many of these patients had other risk factors for chronic renal disease, such as diabetes mellitus or hypertension. In the absence of renal biopsy, which is a limitation of our study, we can only speculate about why these patients did not have uniform improvement or normalization of renal function.

There are some reports before describing sirolimus-based immunosuppressive regimens for nephroprotection. Unfortunately, the follow-up of most of the studies was relatively short, often less than 12 months. Therefore, it is uncertain if the initial increase in creatinine clearance will be sustained over a longer time. However, all 3 of these randomized trials showed improvement in renal function after conversion to sirolimus and thus confirm our results. In our patients, we found that renal function, assessed by serum creatinine level or GFR, improved with discontinuation of CNI administration and replacement by sirolimus therapy. Nine of our 12 patients showed an improvement and 3 further patients showed a stabilization of renal function. In a multicenter study with short observation periods, De Simone and associates investigated the benefit of a switch of immunosuppressive therapy to everolimus with reduction or discontinuation of CNI dosage. Whereas everolimus conversion was safe and without major complications, the primary endpoint of the study (8 mL/min difference in the change in creatinine clearance) was not achieved. One reason could be a rather late switch to everolimus (3 years after liver transplant).

Both sirolimus and everolimus may enhance the nephrotoxicity of cyclosporine. Other studies comparing sirolimus directly with cyclosporine have shown a renal function benefit with sirolimus-based, cyclosporine-free maintenance therapy. A similar enhancement of CNI nephrotoxicity has been reported when sirolimus is combined with tacrolimus. Minimizing CNI when combined with mTOR inhibitors is an area of intense interest, but it is unknown whether these strategies will produce a benefit in renal function as good as early CNI withdrawal or totally CNI-free therapy.

Mulay and associates pointed out, in a systematic review of 5 randomized trials (n=104 patients) and 25 nonrandomized studies (n=977 patients), that a withdrawal of CNI therapy alone is associated with improved creatinine clearance, both in stable patients with kidney transplant and in patients with chronic allograft nephropathy.

The results from 5 multicenter studies indicate that patients receiving sirolimus immunotherapy without cyclosporine have a lower incidence of cancer than patients receiving sirolimus in combination with cyclosporine. Several reports suggest that renal transplant recipients receiving cyclosporine-based immunotherapy may be at increased risk of cancer compared with those whose treatment does not include cyclosporine. Glover and associates found that patients receiving cyclosporine, azathioprine, and prednisolone had a higher incidence of squamous cell skin carcinoma than patients receiving azathioprine and prednisolone without cyclosporine. Similarly, Jensen and associates showed that kidney transplant recipients receiving triple immunosuppression with cyclosporine, azathioprine, and prednisolone had 4.2 times greater risk of cutaneous squamous cell carcinoma than did those receiving azathioprine and corticosteroids. Bedani and associates suggested that cyclosporine may damage the immunologic function of the epidermal Langerhans cells, rendering renal transplant recipients susceptible to Kaposi sarcoma. The risk of posttransplant skin cancer may be related to the degree and duration of long-term immunosuppressive therapy. Dantal and associates reported a significantly higher incidence of cancer in patients receiving normal-dose cyclosporine compared with those receiving low-dose cyclosporine. A retrospective review of the occurrence of malignant neoplasms in renal transplant recipients receiving long-term immunosuppression showed that for patients receiving cyclosporine, the mean for developing the first malignant neoplasm was 4.4 years, whereas for patients not receiving cyclosporine, the mean duration was 9.3 years. Soulillou and Giral reviewed the incidence of infection and cancer in renal transplant recipients, especially in association with immunosuppressants such as cyclosporine. They found that maintenance immunosuppression might be reduced in some renal transplant recipients without adverse effects on patient and graft survival, thus lowering the risk of malignant neoplasm. Furthermore, they suggested that immuno-
suppressive agents might be associated with different risks of cancer.

In a recent study, Campistol and associates\textsuperscript{45} found that at 5 years, the median time to a first skin carcinoma was delayed (491 vs 1126 days), and the risk of a skin carcinoma was significantly lower with sirolimus therapy. Patients who received sirolimus-based, CNI-free therapy after withdrawal of cyclosporine therapy at month 3 had a reduced incidence of both skin and nonskin malignant neoplasms 5 years after renal transplant compared with those who received sirolimus therapy combined with cyclosporine.\textsuperscript{45} Kneteman and colleagues\textsuperscript{15} reported a prospective pilot study of 40 patients with hepatocellular carcinoma treated with a sirolimus-based protocol after transplant. Categorizing recipients within the Milan criteria or beyond, the authors concluded that both 1-year survival and 4-year survival were not significantly different between groups. In a prospective study of 97 patients who received liver transplant because of end-stage liver disease and hepatocellular carcinoma, Zimmerman and associates\textsuperscript{46} found higher overall survival at 1 and 5 years after liver transplant for patients treated with sirolimus (95.5% and 78.8%) versus recipients treated with CNI-based regimens (83% and 62%).

Sirolimus therapy may be associated with adverse events that could greatly affect the quality of life. In a large retrospective series of 175 patients, Montalbano and associates\textsuperscript{17} described bilateral lower extremity edema (57.1%), dermatitis (25.3%), oral ulcers (24.2%), joint pain (23%), pleural effusion (16.5%), increased abdominal girth (5.5%), general edema (5.5%), pericardial effusion (5.5%), facial edema (2.2%), and upper extremity edema (1.3%). Dyslipidemia was reported in up to 44% of patients.\textsuperscript{12, 47, 48}

In 4 of our patients, slight adverse effects (thrombocytopenia, gingival hyperplasia, oral ulceration) occurred. Only 1 patient had to have sirolimus therapy withdrawn because of acneiform rash, and treatment was converted to everolimus. The lower rate of adverse events in our population was probably due to lower sirolimus trough levels than in other reports. Whereas adverse effects such as changes in serum lipids are moderate in our study and could be managed by lipid-lowering agents, Stephany and associates\textsuperscript{49} found that de novo sirolimus-based immunosuppression is associated with a higher frequency of semiquantitative proteinuria; however, estimated graft function at 1 year after transplant remains superior to that of CNI-treated patients. In our study, we also observed a mild elevation of proteinuria, which was not associated with impaired renal function or hypoalbuminemia and was never manifest clinically. The same observations were made by Harper and associates.\textsuperscript{47} The mechanisms and clinical significance of sirolimus-induced proteinuria are poorly understood, particularly in liver transplant. In renal transplant, sirolimus-induced proteinuria seems to be reversible, but evolution to the nephrotic syndrome and deterioration in renal graft function have been reported elsewhere.\textsuperscript{50}

In conclusion, our data suggest that 75% to 85% of liver transplant recipients with renal insufficiency administered CNIs may have sustained improvement in renal function with minimal toxicity when switched to sirolimus therapy. Sirolimus monotherapy appears to provide adequate immunosuppression with a low incidence of acute cellular rejection. Moreover, no patient had acute cellular rejection with sirolimus monotherapy. Adverse effects of sirolimus were mild, requiring dosage reduction in 4 patients and discontinuation of this regimen in 1 patient. Sirolimus use in liver transplant is safe, and in selected patients it is likely to improve significantly long-term outcome.

References
