ORIGINAL ARTICLE

Immunosuppressive treatment with everolimus in patients after liver transplant: 4 years of single-center experience

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KEY WORDS

everolimus, hepatocellular carcinoma, liver transplant

ABSTRACT

INTRODUCTION Everolimus after liver transplant (LT) has been used to minimize the use of calcineurin inhibitors (CNIs), optimize renal function, and prevent recurrence of hepatocellular carcinoma (HCC).

OBJECTIVES We aimed to analyze a single-center experience with switching from CNIs to everolimus in immunossupressive treatment of LT recipients.

PATIENTS AND METHODS A total of 108 LT recipients (men, 65.7%; mean [SD] age, 53.2 [11.1] years) were prospectively enrolled into the study. In all patients, everolimus and CNIs were introduced (target trough levels of 3 to 6 ng/ml and 3 to 5 ng/ml, respectively). After 3 months, CNIs were discontinued in patients who tolerated everolimus well, while everolimus dosage was increased (blood trough levels, 6–12 ng/ml).

RESULTS Everolimus monotherapy was introduced in 32 patients (29.6%), while a combination therapy with everolimus and CNIs was continued in 76 patients (70.4%). However, during a mean follow-up of 27 months (range, 4–50 months), everolimus was withdrawn in 25 patients (33%) due to side effects. In the everolimus-monotherapy group, all patients continued the therapy (P <0.005), but dyslipidemia was more frequent than in patients receiving everolimus and CNIs (40.6% vs 14.5%; P <0.03). Creatinine levels improved significantly in both groups: combination therapy, from 1.58 mg/dl to 1.24 mg/dl after 3 months, and everolimus monotherapy, from 1.19 mg/dl to more than 1 mg/dl. Renal function in the everolimus group was better than in the combination-therapy group (P <0.04). Recurrence of HCC was observed in both groups: combination therapy (9/46 [19.6%]) and everolimus monotherapy (1/17 [5.9%]; P <0.01).

CONCLUSIONS This study showed that switching from CNIs to everolimus after LT allowed a safe weaning of CNIs and an improvement in creatinine levels. In patients on everolimus monotherapy, we observed dyslipidemia as a dose-dependent side effect of the drug as well as a lower risk of HCC recurrence.

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INTRODUCTION Since the first successful liver transplant (LT) in 1967, performed by Starzl, there have been numerous advancements to surgical techniques used in LT as well as to immunosuppressive treatment after LT.[1] Calcineurin inhibitors (CNIs) are considered to be the main immunosuppressive therapy in all solid organ transplants, including LT. In the long term, CNIs can be used as a monotherapy or they can be combined with a mycophenolic acid compound in an attempt to reduce the CNI dose or increase the immunosuppressive potential. The main challenge in CNI treatment is the presence of numerous side effects, including renal failure, cardiovascular complications, and increased risk of malignancy.[2] Another challenge is the risk of hepatocellular carcinoma (HCC) recurrence in cases where HCC was an indication

WHAT'S NEW?

Since the introduction of the mammalian target of rapamycin inhibitor everolimus for immunosuppressive therapy, there are still no specific indications for its use in individual clinical situations or recommendations on the therapeutic dosage after liver transplant (LT). We aimed to present our own experience in terms of therapy switching, long-term outcome, and safety of everolimus treatment after LT. To the best of our knowledge, this is the first such study in Poland. This real-life registry showed that conversion from a calcineurin inhibitor (CNI) to everolimus after LT allowed a significant weaning of the CNI dose and an improvement in creatinine levels shortly after LT, with a low risk of liver graft rejection. Additionally, it revealed dyslipidemia as the main dose-dependent side effect of everolimus therapy as well as a lower risk of hepatocellular cancer recurrence in LT recipients treated with everolimus as monotherapy, which probably resulted in improved prognosis.

TABLE 1 Time intervals between liver transplant and administration of the first everolimus dose (n = 108)

Time interval, d	No. (%) of patients
<30	8 (7.4)
30–90	52 (48.2)
90–180	17 (15.7)
>180	31 (28.7)

for LT, regardless of whether the patient meets the Milan criteria.[3]

In recent years, the mainstay of immunosuppressive treatment modifications is a reduction of CNI dosage in an attempt to avoid as many side effects as possible. Another important aim is to reduce the risk of HCC recurrence in patients undergoing LT due to liver cancer. The mammalian target of rapamycin (mTOR) kinase inhibitor everolimus is a modern immunosuppressive drug. However, it is unclear when and how LT recipients could benefit from this treatment. A positive impact of everolimus on renal function with a simultaneous reduction of the CNI dose (or even everolimus alone with mycophenolate mofetil) has been shown, together with the potential to prevent HCC recurrence.[2] However, in most pharmacological trials, a complete withdrawal of CNIs or a significant dose reduction was associated with an increased risk of acute rejection.[2,4]

In the present study, we describe our single-center experience in terms of therapy switching, long-term outcome, and safety of everolimus treatment.

PATIENTS AND METHODS From February 2013 to May 2017, 679 LTs from deceased donors were performed in the Central Clinical Hospital of the Medical University of Warsaw, Poland. Of this group, 108 patients were prospectively included in an observational study with everolimus as immunosuppressive therapy. The study design was reviewed by the Ethics Committee of the Medical University of Warsaw. The committee decided that no additional patient consent was required to analyze the treatment data because the drugs used in the study are all fully approved.

Patients were included into the study according to the criteria proposed by De Simone et al.[5] The most common indications for modification of immunosuppressive therapy were similar to those in other published studies and included impaired renal function, LT due to HCC (regardless of whether a patient fulfilled the Milan criteria or an expanded set of criteria proposed by the University of California San Francisco or up-to-seven criteria, which are used at our center), and intolerance of CNIs.[2,6] This was not a randomized controlled trial. It was an observational study assessing real-life patients recruited at the time when the main indications were established for the modification of immunosuppressive regimen to include the mTOR inhibitor everolimus as a leading therapy. Therefore, the time between LT and the first dose of everolimus was different in each patient, and the immunosuppressive treatment with everolimus was standardized from the moment of taking the first everolimus dose according to the above treatment protocol. The time intervals between LT and the first everolimus dose are presented in TABLE 1.

All patients included in the study underwent LT using the piggyback technique. In the perioperative period, all patients received induction treatment with intravenous basiliximab (2 × 20 mg; the first dose in the anhepatic phase, and the second, on day 4 after LT). Before enrollment, immunosuppressive therapy was based on tacrolimus as the main drug at a dose that allowed maintaining the mean through level of 7 to 10 ng/ml. In addition, all patients received mycophenolate mofetil (MMF) at a starting dose of 2 × 500–1000 mg (a higher dose was usually used in patients with clinical features of renal failure to allow a reduction of the tacrolimus dose to the drug concentration in blood of 5 to 7 ng/ml). They also received prednisone at a starting dose of 20 mg/d tapered by 5 mg every 3 weeks until discontinuation at 12 weeks. Mycophenolate mofetil was reduced to a dose of 2 × 250 mg or even discontinued if significant leukopenia or diarrhea occurred. The decision was at the discretion of the consultant physician during follow-up visits at an outpatient clinic for LT recipients.

On the day of inclusion in the study, each of the 108 patients was administered half of the current tacrolimus dose (to achieve a blood concentration level of 3–5 ng/ml) and was started on everolimus at a dose of 1 mg twice daily (at a drug concentration in blood maintained at 3–6 ng/ml). The regimen was maintained for 3 consecutive months and then modified depending on the tolerance of therapy. In patients who tolerated everolimus well, tacrolimus was discontinued and the everolimus dose was increased to maintain blood trough levels of 6 to 12 ng/ml. The combined treatment with everolimus and tacrolimus was maintained in patients in whom the side effects of everolimus significantly worsened after

TABLE 2 Characteristics of the study group (n = 108)

Parameter		Value
Age, y, mean (SD)		53.2 (11.1)
Sex, male/female, n (%)		71/37 (65.7/34.3)
Main indication for LT, n (%)	HCC	61 (56.5)
	Cirrhosis due to HCV infection	61 (56.5)
	Cirrhosis due to HBV infection	27 (25.0)
	Autoimmune/biliary cirrhosis	20 (18.5)
	Alcoholic liver disease	17 (15.7)
	Other malignant liver tumors	1 (0.9)
Retransplantation, n (%)		4 (3.7)

Abbreviations: HBV, hepatitis B virus; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; LT, liver transplant

TABLE 3 Clinical conditions that led to everolimus therapy in the study group (n = 108)

Indication for everolimus therapy	Everolimus (n = 32)	Everolimus + CNI (n = 76)
Prevention of HCC recurrence	17 (53.1)	46 (60.5)
Chronic renal failure	15 (46.9)	33 (43.4)
CNI intolerance[a]	8 (25.0)	5 (6.6)
Recurrent HCC	1 (3.1)	9 (11.8)
Other malignancy	1 (3.1)	4 (5.3)

Data are presented as number (percentage) of patients.

a CNI intolerance: neurotoxicity (including severe headaches unresponsive to pharmacotherapy and posterior reversible encephalopathy syndrome) or worsening renal function following the introduction of CNI treatment due to liver transplant

Abbreviations: CNI, calcineurin inhibitor; others, see TABLE 2

an attempted switch to everolimus monotherapy at a higher dose or in whom the side effects occurred during the first 12 weeks of therapy but their range and severity was acceptable to the patient and physician. One of the most common complications of everolimus therapy, hyperlipidemia, was treated by a 50% reduction of the everolimus dose as the first-line treatment and statin or fibrate pharmacotherapy as the second-line treatment.

Patients who had undergone LT due to HCC were included in a screening program according to the European Association for the Study of the Liver and European Organisation for Research and Treatment of Cancer guidelines.[7] Abdominal ultrasonography and the measurement of α-fetoprotein levels were performed every 4 to 6 months. If HCC recurrence was suspected, imaging studies such as abdominal contrast-enhanced computed tomography or magnetic resonance imaging as well as thoracic high-resolution computed tomography were performed. The recurrence of HCC and its further course were recorded each time in an internal registry of the transplant center. The recurrence was not an exclusion criterion in the study.

Statistical analysis Owing to the nonparametric data distribution with a tendency for normal data distribution, the Shapiro–Wilk test as the main statistical test and Kolmogorov–Smirnov test with the Lilliefors correction as an additional test were used. Differences between groups (everolimus vs everolimus and tacrolimus) were analyzed using the Mann–Whitney test. The Fisher exact test for small-group analyses was used. The results were presented as means with standard deviations. A P value of less than 0.05 was considered significant.

RESULTS The study group included 108 patients (men, 65.7%; women, 34.3%; mean [SD] age, 53.2 [11.1] years). A history of liver damage caused by hepatitis B or C virus infection was reported in 62% of the patients, HCC was diagnosed in 58.3% of the patients, and 44.4% of the patients had documented clinical features of renal failure. The mean follow-up was 27 months (range, 4–50 months). The mean (SD) duration of everolimus treatment in the whole study group was 27 (13) months. After the first 12 weeks of therapy, treatment with CNIs was discontinued in 32 of the 108 patients (29.6%). These patients continued everolimus treatment at a serum concentration of 6 to 12 ng/ml. The remaining 76 patients (70.4%) were not switched to everolimus monotherapy as the main treatment, mainly due to the severity of adverse drug reactions, including 25 patients (33%) who stopped everolimus altogether in consultation with the treating physician during follow-up outpatient visits. During the follow-up, everolimus was discontinued in 25 of 108 patients (23.1%) due to severe side effects of the drug (mainly diarrhea and skin reactions with pruritus). The detailed characteristics of the study subgroups and indications for everolimus therapy are shown in TABLES 2 and 3.

Patients on everolimus monotherapy (although all of them continued long-term treatment unlike patients on combination therapy; $P < 0.005$) were more likely to have lipid disorders (hypertriglyceridemia and hypercholesterolemia) than those treated with a combination of everolimus and CNIs (40.6% vs 14.5%; $P < 0.03$).

Renal function assessed by regular monitoring of blood creatinine levels significantly improved in both groups only after 3 months of therapy. The mean blood creatinine level in patients on combination therapy was 1.58 mg/dl at baseline, as compared with 1.19 mg/dl in the everolimus-monotherapy group. At 3 months, the mean blood creatinine level dropped to 1.24 mg/dl in the combination-therapy group and to 1 mg/dl in the everolimus-monotherapy group ($P < 0.04$). The levels were further reduced at 12 months, with 1.06 mg/dl observed in the combination-therapy group and 0.94 mg/dl in the everolimus-monotherapy group. However, the difference was no longer significant ($P = 0.12$).

In our study, 63 patients (58.3%) underwent LT due to HCC. During the follow-up, the recurrence of HCC was confirmed in 10 patients (15.9%). The mean (SD) follow-up of patients with HCC was 20.3 (25.5) months. The distribution

TABLE 4 Side effects of everolimus therapy (n = 108)

Side effects	Everolimus + CNI (n = 76)	Everolimus (n = 32)	*P* value
Lipid disorders	11 (14.5)	13 (40.6)	0.003
Skin rash/pruritus	14 (18.4)	6 (18.7)	0.28
Diarrhea	9 (11.8)	4 (12.5)	0.98
Peripheral edema	12 (15.8)	7 (21.9)	0.51
Normocytic anemia	11 (14.5)	2 (6.2)	0.21

Data are presented as number (%) of patients.

Abbreviations: see TABLE 3

of patients with HCC in the study groups was proportional: 17 of the 32 patients (53%) in the everolimus-monotherapy group and 46 of the 76 patients (61%) in the combination-therapy group. The recurrence of HCC was more common in patients on combination therapy than in those on everolimus monotherapy (with higher everolimus trough levels): 9 of the 46 patients (19.6%) and 1 of the 17 patients (5.9%), respectively ($P < 0.01$).

The most frequent side effects of everolimus therapy, in addition to lipid disorders described above, were skin lesions (similar to those seen in atopic dermatitis or urticaria), watery diarrhea, peripheral edema, and normocytic anemia. The number of individual complications (with the exception of lipid disorders) did not differ significantly between groups. Data on the number of complications associated with everolimus therapy during follow-up are presented in TABLE 4.

During the follow-up, none of the patients had a clinically suspected or histologically proven episode of acute liver graft rejection, problems with wound healing, or any episodes of hepatic artery thrombosis. The overall survival rate of LT recipients was 83.3%.

DISCUSSION It has been several years since the new mTOR inhibitor everolimus was introduced into immunosuppressive therapy. However, there are still no specific indications regarding its application in individual clinical situations or recommendations on the therapeutic dosage, despite frequent reports on its use in organ transplant recipients. However, a few authors investigated the effects of everolimus therapy after LT. Therefore, we aimed to present our own experience in terms of using everolimus in LT recipients. To the best of our knowledge, this is the first such study conducted in Poland.

Our analysis showed that less than one-third of the patients tolerated everolimus at a higher dose that would allow a discontinuation of tacrolimus, and nearly one-fourth of the patients were unable to continue treatment due to the severity of adverse drug reactions. These results are in line with those presented by De Simone et al[8] and Rodriguez-Peralvarez et al[9] (discontinuation rate of 23% and 25%, respectively). The most frequent side effects of everolimus therapy were lipid disorders, skin lesions, watery diarrhea, peripheral edema, and normocytic anemia. Lipid disorders were more common in patients on everolimus monotherapy than in those on combination therapy. These findings are in line with the study by Levy et al,[10] who reported a relationship between the severity of lipid disorders and the dose of everolimus. A recent multicenter trial by Cillo et al[11] noted a similar occurrence of lipid disorders in patients treated with everolimus. However, in contrast to our study, they reported no significant problems with postoperative wound healing. Yet, it is important to note that the vast majority of our patients had their treatment modified to include everolimus a few months after LT.

Interestingly, the disturbances in blood lipid levels caused by everolimus do not seem to have an equally negative impact on the cardiovascular risk in LT recipients as CNIs have on worsening renal function. This issue was addressed by Saliba et al,[12] who reported a significantly lower risk of cardiovascular incidents in LT recipients who were put early on everolimus treatment, as compared with the same group of patients who received CNI therapy. According to the authors, this was due to cardiac burden associated with chronic renal failure as a result of long-term use of higher CNI doses.

During the follow-up, none of the patients in our study had a clinically suspected or histologically proven episode of acute liver graft rejection. De Simone et al[8] reported that nearly 5% of all patients on everolimus monotherapy had a histologically confirmed acute graft rejection (based on a previous clinical suspicion).[8] In other reports, the risk of biopsy-proven acute rejection ranged considerably from approximately 1% to 15%.[4-6,13,14] The discrepancy may result from the fact that in our center all patients who underwent LT due to HCC or liver insufficiency associated with renal failure receive intravenous anti–interleukin-2 receptor antibodies (according to an internal posttransplant immunosuppressive treatment protocol), which certainly translates to improved control of the acute graft rejection process. Our observations are in line with those reported by Cholongitas et al.[15] In addition, our patients were treated with MMF during CNI withdrawal, which had significantly reduced the risk of acute graft rejection, thus improving the safety of therapy. Similar favorable effects of MMF in LT recipients after switching from a CNI to an mTOR inhibitor were reported by Saliba et al[4] and De Simone et al.[14]

Although data on the impact of immunosuppressive treatment with everolimus on renal function are quite clear, there have been some conflicting reports.[2,6,16,17] Recent pharmacological studies, complemented by histopathologic findings, reported that the characteristic damage of tubular structures seems to be involved in kidney function impairment during CNI treatment, and switching to an mTOR inhibitor is particularly effective in renoprotection.[18] In our study, renal

function assessed by regular monitoring of blood creatinine levels (the simplest routine biomarker of renal function in LT recipients used in our center) improved in both study groups only after 3 months of therapy. In the subsequent months of follow-up, the mean serum creatinine levels showed a further tendency to normalize (although no longer with a significant difference between groups). Similar positive results were reported by Cholongitas et al.[19] Although our follow-up was shorter than in the cited papers, our results in a Polish population are in line with those from previous studies. Again, in our study, the introduction of everolimus was associated with a significant improvement of renal function in LT recipients. Possibly, the follow-up duration in our study was too short and the study groups were too small to confirm long-term clinical benefits of immunosuppressive treatment modification, as it was clearly demonstrated by De Simone et al[8] in patients de novo after LT.

It is well known that immunosuppressive therapy after LT for HCC is a risk factor for tumor recurrence. A substantial amount of data from pharmaceutical trials (first with the mTOR inhibitor sirolimus and then with the newer everolimus) show the potential role of mTOR inhibitors in reducing the risk of recurrence due to their antiproliferative effect described in some in vitro cellular models or in vivo studies in LT recipients.[20-22] The most recent studies emphasize the important role of everolimus in reducing the risk of HCC recurrence after LT.[16,19,23] In our study, more than half of the patients were transplanted because of HCC. During the follow-up, HCC recurred more often in patients on combination therapy than in those on everolimus monotherapy. This may lead to a conclusion that patients on everolimus monotherapy in whom higher everolimus through levels were obtained as per the study design, the risk of HCC recurrence is lower than in those with lower everolimus trough levels. Our findings are in line with the study by Cholongitas et al.[24] These results seem to be optimistic also considering the possibility of using other criteria than Milan for evaluating patients' eligibility for LT (ie, University of California San Francisco or up-to-seven criteria), as allowed in our center. However, further studies on a larger group of patients are needed to confirm these observations.

Sailba et al[17] reported contrasting results regarding HCC recurrence, despite a similar number of patients undergoing LT due to HCC. They did not reveal any differences in HCC recurrence after LT between patients on everolimus monotherapy and those on combination therapy with everolimus and tacrolimus at lower doses. Yet other studies did not observe any cases of HCC recurrence after LT.[6] The discrepancies between studies may result from the fact that in our center, patients who do not meet the Milan criteria are also eligible for LT. These findings suggest that switching the immunosuppressive therapy from CNIs to mTOR inhibitors might be particularly important in patients with HCC who underwent a transplant according to extended eligibility criteria for LT. This is also in line with recent studies by Thorat et al[25] and Ferreiro et al.[26] It is possible that, ultimately, we will be able to develop precise criteria for identifying HCC patients after LT who will benefit most from immunosuppressive treatment with mTOR inhibitors instead of CNIs, as recently suggested by Rodriguez-Peralvarez et al.[9] Based on our results and emerging reports from other centers, an immunosuppressive protocol including everolimus in combination with kinase inhibitors (eg, sorafenib or regorafenib) might be an interesting therapeutic option in patients with HCC recurrence after LT. However, this issue requires further studies.[27]

In summary, immunosuppressive therapy with everolimus after LT is safe and is associated with a low risk of acute liver graft rejection and good long-term outcomes in terms of graft function, especially in patients after LT for complications of viral hepatitis, including HCC. Introduction of everolimus allows a reduction of the tacrolimus dose or even its discontinuation in the majority of patients, which may improve renal function. Treatment with everolimus at higher blood levels might reduce the risk of HCC recurrence after LT, but this requires further studies on a larger population, with a longer follow-up, and a highly selected group of patients after LT due to HCC. The most common reasons for failure of everolimus treatment are side drug reactions, the severity of which is usually proportional to the drug blood level. Attention should be paid to lipid metabolism disorders, which might require additional pharmacotherapy. Our observational prospective study and the available literature suggest that everolimus might be a beneficial therapeutic option for a selected group of LT recipients after a careful consideration of its potential side effects.

ARTICLE INFORMATION

CONTRIBUTION STATEMENT MPW contributed to the study design, writing and editing of the article, data analysis and interpretation, final revision of the manuscript, and the discussion of the content. JR-W contributed to manuscript editing and data analysis. MJ contributed to statistical analysis. All authors contributed to data collection and patient care.

CONFLICT OF INTEREST None declared.

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