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The efficacy and safety of mammalian target of rapamycin inhibitors *ab initio* after liver transplantation without corticosteroids or induction therapy

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ABSTRACT

Background: Mammalian target of rapamycin inhibitors have been used along with corticosteroids and/or induction therapy immediately after liver transplantation. Our aim was to assess the safety and tolerability of everolimus *ab initio* after liver transplantation without corticosteroids or induction, as well as efficacy in terms of liver function, rejection and graft loss.
Methods: A retrospective observational study of 50 adult patients (86% males, median age 54 years, range 25–68) who were liver transplanted between 2009 and 2013 and followed for 12 months. All recipients received everolimus plus low doses of calcineurin inhibitors ($n = 38$) or mycophenolate ($n = 12$) without corticosteroids and/or induction from the day of transplant.
Results: The overall patient and graft survival was 80%. Liver function was stable during one year follow-up. No rejections or graft loss were observed. Only five patients (10%) required therapy for onset dyslipidemia.
Conclusion: Everolimus-based immunosuppression regimen without corticosteroids and/or induction immediately after liver transplantation seems to be safe and effective when administered with low doses of calcineurin-inhibitor or mycophenolate; although these findings require further investigation, these regimens could avoid adverse effects of standard immunosuppression regimens with higher doses.

1. Introduction

Immunosuppressive regimens following liver transplantation (LT) usually involve calcineurin inhibitors (CNIs) combined with steroids and/or other immunosuppressive drugs, such as mycophenolate or mammalian target of rapamycin inhibitors (mTORi) [1]. However, it is well known that exposure to CNIs is associated with dose-dependent short and long-term side effects such as nephrotoxicity, cardiovascular disease, new-onset diabetes mellitus, and hyperlipidemia [2,3]. Renal dysfunction is the most frequent and well-recognized side effect and may occur as an acute impairment due to the vasoconstriction of renal arterioles (which can be reversed by reducing or withdrawing administration of CNIs) or as a chronic impairment with irreversible structural damage, leading to end-stage renal disease in up to 20% of patients [4,5]. Therefore, the current clinical goal remains the same: namely, to identify a tailored immunosuppressive regimen with the aim of avoiding rejection while minimizing the side effects.

The use of mTORi in the LT setting has been mostly adopted after early (<3 months of LT) or late (>3 months) conversion from CNIs [6–8]. Most studies on everolimus, a proliferation–signal inhibitor with anti-proliferative and immunosuppressive activity [9,10], have shown that the reduction [11] or withdrawal [12] of CNIs is feasible without loss of efficacy; these studies have demonstrated that everolimus allows the discontinuation or at least a major reduction of CNI dose, however an early conversion is required to improve renal function after LT. In this regard, only a small number of studies have investigated the early use of everolimus after LT [11,13–18] and only one phase-2 trial has demonstrated the safety and efficacy of everolimus from the first post-transplant day in association with low dose CNIs and corticosteroids [19].

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Herein we report on the safety and efficacy of everolimus use *ab initio* after LT, in combination with low dose CNIs or mycophenolate without corticosteroids or induction therapy.

2. Materials and methods

2.1. Study design

We designed a retrospective observational study to evaluate the effects of immunosuppressive treatment based on everolimus *ab initio* after LT. All patients who were liver transplanted at our institution and who were treated with everolimus in association with mycophenolate or CNIs *ab initio* were enrolled.

The study population included all adult recipients who received a graft from deceased donors. The decision to use everolimus -based immunosuppressive therapy with low dose CNIs was taken on the basis of the following indications: hepatocellular carcinoma (HCC) within Milan criteria after down-staging ($n = 12$) to minimize post-transplant risk of CNI-related dose-dependent HCC recurrence [20]; for hepatitis C virus (HCV)-related cirrhosis ($n = 15$) to decrease CNI dose to avoid early post-LT HCV recurrence [21]. mTORi use with low dose CNIs was also considered in patients who had a history of insulin-dependent diabetes mellitus (IDDM; $n = 6$), neoplasm ($n = 4$) or hypertension ($n = 1$) before transplant. Moreover, everolimus -based immunosuppressive therapy with anti-metabolite was considered whenever LT patients showed renal impairment before surgery (*i.e.* on the waiting list) or in those recipients who had predictive factors for post-transplant renal impairment (*i.e.* haemodynamic instability during anaesthesia, intraoperative bleeding, high volume of transfused bloods products). Treatment was started from the first post-operative day (POD 1). Pre-existing renal impairment with estimated-glomerular filtration rate (eGFR) <30 ml/min/1.73 m^2 calculated using the four-variable modification of diet in renal disease (MDRD) [22] formula was not considered an exclusion criteria for the study. Patients who had had a previous LT or who underwent a simultaneous liver and kidney transplantation were not included in the study.

The study was approved by the institutional ethics committee and all patients provided written consent to data collection.

2.2. Study endpoints

The primary objective was to assess the midterm safety of everolimus use immediately following LT without the use of corticosteroids or induction therapy. The secondary objective was to evaluate efficacy in terms of liver function, graft loss and the incidence of acute and chronic rejection during follow-up.

2.3. Graft function and liver histology evaluation

Graft function was assessed *via* standard liver function tests (LFTs) [including total bilirubin, direct bilirubin, aspartate aminotransferase (AST), alanine aminotransferase (ALT), gamma-glutamyl-transferase (gamma-GT), albumin], from the time of LT until the last follow-up visit. Laboratory tests (such as complete blood count and biochemistry tests) and physical examination were performed before LT, during the first week post-LT, at POD 15 and at months 1, 3, 6, and 12 post-LT.

In all recipients, liver biopsies were carried out every time the physical and laboratory data were suggestive of graft rejection. Furthermore, recipients with HCV infection underwent protocol liver biopsies at 6 months and 1 year post-LT. Histological examinations were carried out by experienced pathologists. Acute rejection was defined according to standard criteria and chronic rejection was assessed according to Banff classification rejection activity index (RAI) [23]. Biopsies were scored for grade of necro-inflammatory activity and stage of fibrosis according to the Ishak scale [24]. Liver specimens were obtained percutaneously using 1.6 mm modified Menghini needles. To minimize sampling errors, only specimens that were longer than 1.5 cm and wider than 1.4 mm were considered, including ≥8 portal tracts [25]. Specimens were formalin-fixed and paraffin-embedded for histological analysis. The 5-ìm sections were stained for haematoxylin and eosin, and Masson's trichrome stain for collagen and cytokeratins for the assessment of ductopenia.

2.4. Renal function and everolimus measurement

Change in renal function was assessed by estimated eGFR using the MDRD formula [22] prior to LT, during the first post-operative week, and at 1, 3, 6 and 12 months post-LT. Measurement of everolimus levels in blood was assayed on a LC–MS/MS analyzer [26].

2.5. Statistical analysis

Data were collected retrospectively from a prospective database (Microsoft Access 2.0, Microsoft Corporation, USA). Categorical variables were analyzed using Fisher's exact test (F test). The normal continuous data were analyzed using a parametric test (t test). Statistical results were expressed as median and range. A p-value of <0.05 was considered significant. The programme used for statistical analysis was SPSS® 13.0 (233 South Wacker Drive, Chicago, USA) for Mac.

3. Results

3.1. Patient population and survival

Between September 2009 and November 2013, 108 patients received LT at our institution; 50 non-consecutive patients (86% males, median age 54 years, range 25–68) received everolimus-based therapy and were included in the present study; 58 patients were excluded for the following reasons: 45 were treated with tacrolimus once daily and mycophenolate mofetil (MMF) in accordance with another multicentre study; 5 received a combined liver and kidney transplant; 8 were treated with CNIs.

Characteristics of the study population are summarized in Table 1.

Of the 50 patients, 40 (80%) were alive and in good clinical condition at of 12 months' follow-up. The causes of death are summarized in Table 2. The one-year patient and graft survival was 80% (Fig. 1).

3.2. Immunosuppression regimen

The immunosuppression regimen was everolimus -based in combination with CNIs or mycophenolate from the 1st POD after LT. The aim was to achieve target blood everolimus levels of 3–8 ng/ml within the first week. The CNI target level was 3–5 ng/ml in the tacrolimus group and 500–700 C_2 in patients who received cyclosporine (CsA). As per our institution' policy, no patient received steroids or induction therapy.

The starting median dose of everolimus was 1.5 mg/day (range 0.5–1.5) without an initial loading dose. The median everolimus daily doses at 1, 3, 6, 12 months post-LT were 2.0 mg/day (range 0.5–2.5), 1.0 mg/day (range 0.5–1.5), 1.0 mg/day (range 0.5–1.5), and 2.0 mg/day (range 0.75–2.5), respectively. At 1 month post-LT, the median everolimus blood concentration was 4.0 ng/ml (range 2.2–9.2) and no significant variation of trough level was observed during the entire observational period ($p = 0.12$, Fig. 2).

Thirty patients (60%) received everolimus with tacrolimus once daily. The starting median daily dose of tacrolimus was 3 mg/day (range 3–5, given qd in the morning) to achieve a trough level