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ORIGINAL ARTICLE

Treatment with mTOR inhibitors after liver transplantation enables a sustained increase in regulatory T-cells while preserving their suppressive capacity

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KEYWORDS
mTOR inhibitor;
Liver;
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Regulatory T-cells;
Calcineurin inhibitor.

Summary
Background: The mammalian targets of rapamycin (mTOR) inhibitors (sirolimus [SRL] and everolimus [EVR]) are used after transplantation for their immunosuppressive activity. Regulatory T-cells (Tregs) play a crucial role in immune tolerance. mTOR inhibitors appear to preserve Tregs, unlike Tacrolimus (Tac).
Aim: The aim of this study was to evaluate the number and function of Tregs in liver transplant recipients before and after conversion from Tac to mTOR inhibitors.
Methods: Fifteen patients with stable graft function where converted to SRL ($n = 5$) or EVR ($n = 10$). Tregs ($CD4^+$ $CD25^+$ $FoxP3^+$ $CD127^{low}$) number and activity were analysed prospectively in blood cells using flow cytometry, and functional assay.
Results: Patients of both groups displayed a sustained rise in Treg levels after introduction of mTOR inhibitors (Treg levels at 3 months: $6.45 \pm 0.38\%$ of CD4 T-cells, vs. baseline level

Abbreviations: CD, Cluster of differentiation; CNI, Calcineurin Inhibitor; EVR, Everolimus; HCC, Hepatocellular carcinoma; MMF, Mycophenolate Mofetil; PBMC, Peripheral Blood Mononuclear Cell; PTLD, Post-Transplant Lymphoproliferative Disease; PSC, Primary Sclerosing Cholangitis; SRL, Sirolimus; Tac, Tacrolimus; Tregs, Regulatory T-cells.

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of 3.61 ± 0.37%, $P < 0.001$; mean fold increase 2.04 ± 0.73). In SRL group, 3-month Treg levels were 6.01 ± 0.53 vs. 3.79 ± 0.39; $P = 0.037$, while in EVR group they were 6.63 ± 0.67 vs. 3.54 ± 0.51; $P = 0.001$. By contrast, no statistical change was observed in an unconverted Tac control group. Tregs also preserved their functional ability to suppress activated T-cells.
Conclusion: These results suggest that mTOR inhibitors induce a significant increase in Tregs while maintaining suppressive activity after LT.

Introduction

Calcineurin inhibitors (CNI) such as Tacrolimus (Tac), the principal immunosuppressive drugs used after liver transplantation (LT), act by interfering with the activation of calcineurin phosphatase, which in turn triggers nuclear factors. These pathways are critically involved in T-cell activation. Despite the fall in rejection rates, the side effects of the long-term CNI use are a major adverse outcome [1]. Other immunosuppressive agents such as inhibitors of the mammalian target of Rapamycin (mTOR) have been introduced, but their use remains limited in the context of LT. mTOR inhibitors act by binding to FK-binding protein 12, after which the complex associates with the mammalian target of Rapamycin. This inhibits the mTOR complex that controls crucial biochemical pathways, affecting the organisation of the actin cytoskeleton, mRNA transcription, protein turnover and the initiation of protein translation and synthesis, all of which are required for lymphocyte proliferation and are essential to development of the immune response [2]. Sirolimus (SRL) is an antifungal macrolide and was the first molecule in the mTOR inhibitor group to be discovered; it displays potent anti-proliferative activities that generate anti-tumour and immunosuppressive effects [3]. Everolimus (EVR), a SRL analogue, has been used in the treatment of several types of cancer [4] and to prevent graft rejection [5,6]. The potential ability of mTOR inhibitors to modulate the immune response, in addition to their anti-proliferative activities, may therefore have a beneficial effect on the relapse rate associated with immunosuppression in transplant patients with hepatocellular carcinoma, like shown in a recent randomized controlled trial indicating that mTOR inhibitors have a beneficial impact on HCC-recurrence, and mTOR inhibitors-based immunosuppression was suggested to be associated with lower HCC recurrence after LT in a systemic review [7,8].

The natural Treg population, which accounts for 5—10% of peripheral $CD4^+$T-cells, constitutively expresses CD25 [9], and can suppress host immune responses in a context of autoimmune diseases and transplantation [10]. The FoxP3 transcription factor that is expressed by Tregs appears to be a master gene controlling Treg development [11], and the majority of FoxP3+ Tregs of are CD127 low [12]. Mouse and human Tregs can be expanded *in vitro* using SRL and high-dose IL-2, while inhibiting the proliferation of effector T-cells [13,14]. There is evidence to suggest that Tregs play a crucial role in the mechanism of immune tolerance; indeed, in the post-organ transplant setting, several experimental studies have demonstrated that Tregs can induce allograft tolerance and could play a therapeutic role [15—18]. Tregs are influenced by immunosuppressive therapy; in particular, CNIs impair the Treg function *in vitro* [19]. Unlike CNIs, it is suggested that mTOR inhibitors favour the expansion and immunomodulatory functions of $FoxP3^+$ $CD4^+$ $CD25^+$ Treg both *in vitro* and *in vivo* [14,20,21]. It has also been shown that Treg levels fall significantly after LT, especially in a context of allograft rejection [22], and that the reduction in circulating Tregs is counterbalanced by an increase within the graft [23]. Long-term treatment with CNIs in human renal transplant recipients has appeared to induce a reduction in the number of circulating Tregs, whereas SRL do not seem to do so [24]. These findings point to mTOR inhibitors as being capable of increasing blood Treg levels in patients previously treated with CNIs [25].

During this pilot study, we prospectively monitored the number and function of Tregs in the peripheral blood of fifteen liver transplant recipients who had been switched from a CNI to an SRL or EVR-based immunosuppressive regimen because of the presence of cancer. The prospective evaluation presented herewith shows that conversion from Tac to an mTOR inhibitor (SRL or EVR) induced a sustained and significant increase in peripheral blood $CD4^+CD25^+CD127^{low}FoxP3^+$ Tregs, while at the same time preserving their suppressive capacity.

Patients and methods

Patients and immunosuppressive protocols

Fifteen patients who had received a liver transplant in our centre, and who had been converted from a Tac to an mTOR inhibitor-based immunosuppressive regimen were included consecutively before the conversion in this pilot study. They had all received a Tac-based immunosuppressive regimen with stable graft function, before being converted to an SRL ($n = 5$) or EVR ($n = 10$) immunosuppressive regimen. The rationale for conversion was the presence of a pre-transplant liver tumour confirmed by pathological examination of the explant. This study was approved by the Ethics Committee (CPP) for Ile de France III (AO 1185-38), and all patients gave their informed consent to be included in the study.

All patients received the same immunosuppressive regimen, which included low-dose corticosteroids that were withdrawn between 6 and 12 months post-LT, Tac to maintain trough blood levels at between 8 and 11 ng/ml during the first 12 months post-LT, and then at between 5 and 8 ng/ml, and mycophenolate mofetil (MMF) 1 g bid. The study design is shown in Fig. 1. Conversion protocol to the mTOR inhibitor was performed as follows: following the introduction of SRL 2 mg/day or EVR 1 mg bid, the daily Tac dose was reduced by 50%, and then tapered to complete withdrawal after less

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