EASL EUROPEAN ASSOCIATION FOR THE STUDY OF THE LIVER | JOURNAL OF HEPATOLOGY

m-TOR inhibitors: What role in liver transplantation?

Toshiyasu Kawahara, Sonal Asthana, Norman M. Kneteman*

Division of Transplantation, Department of Surgery, University of Alberta, Canada

The development of calcineurin inhibitors (CNIs) led to marked improvements in patient and graft survival after liver transplantation (LTx). We have been left, however, with a dependence on immunosuppressive agents with nephrotoxicity, neurotoxicity, adverse impacts on cardiac risk profile, and risk for malignancy. These challenges need to be met against a dominance of hepatitis C virus (HCV) and hepatocellular carcinoma (HCC) as indications for liver transplant. Unmet needs for immunosuppression (IS) in LTx include:

(1) Effective drugs that avoid CNIs toxicities.
(2) Agents without adverse impact on HCV recurrence.
(3) Compounds that minimize risk of HCC recurrence.

New immunosuppressives will need to address the above needs while supporting patient and graft survival equivalent to those achievable with CNIs, ideally without important new toxicities. Two new classes of agents are currently in advanced clinical development: belatacept, and the mammalian target of rapamycin inhibitors (m-TORi). This manuscript will review evidence for a role for m-TORi in LTx in a range of clinical scenarios including patients with CNI nephrotoxicity or neurotoxicity, patients at risk of (or with) HCV recurrence, and patients at risk of HCC recurrence.

Introduction

Liver transplantation (LTx) has become a standard therapy for end stage liver disease, including that due to hepatitis C cirrhosis and non-resectable hepatocellular carcinoma (HCC). The outcomes of LTx have improved with advances in surgical procedures and immunosuppressive drugs, especially calcineurin inhibitors (CNIs). However, chronic kidney disease (CKD) caused by CNIs, recurrence of hepatitis C in the transplanted liver, and recurrence of HCC remain major problems after LTx. Renal insufficiency in LTx is associated with progression to end stage renal disease and a decrease in patient and graft survival [1–3]. CNIs have been associated with a dose-dependent increase in the post-transplant risk of HCC recurrence [4]. Minimizing the nephrotoxicity and exploring for anti-tumor effect of immunosuppressive regimens may help to reduce the number of patients developing CKD and HCC recurrence after LTx.

Sirolimus (SRL) and everolimus (EVRL) inhibit mammalian target of rapamycin (m-TOR). The m-TOR is an evolutionarily conserved PI3-kinase family member that plays a key role in integrating different biochemical and growth factor signals, including amino acids, glucose, ATP, and insulin [5]. m-TOR inhibitors (m-TORi) continue to be explored as immunosuppressive drugs in allogeneic transplantation and as novel anticancer agents. In this review article, we will discuss the impact of m-TORi in LTx, with specific reference to the important areas of kidney function, hepatitis C recurrence, and HCC recurrence, and thereby explore the rationale for selective use of m-TORi in liver transplantation.

The first report on use of m-TORi in LTx achieved a modest 67% 1-year survival in 15 patients, 8 of whom had HCC [6]. Early reports by McAlister, Chang, and Trotter supported the potential for SRL-based immunosuppression to achieve outcomes equal to CNI-based protocols [7–9]. After a report of the second international multicenter trial of SRL in LTx, delivered at the American Transplant Congress in 2002, documenting an increase in rate of graft loss and death and a trend to an increase in HAT in the SRL/Tac arm [10], the Food and Drug Administration (FDA) issued a "black box" warning on use of SRL in LTx. As appropriate under the "black box", subsequent studies with SRL have focused on areas and patients where potential adverse impacts from SRL were felt to be outweighed by likely benefit. These include patients with post-transplant nephrotoxicity or neurotoxicity, and patients with hepatocellular carcinoma (HCC), where the anti-tumor impact of m-TORi may prove beneficial. Review of outcomes with m-TORi in these areas of liver transplantation will form the core of this review.

Mammalian target of rapamycin (m-TOR) inhibitors

Rapamycin, also known in clinical usage as SRL, was isolated from a soil sample obtained in Easter Island (Rapa Nui) and was

Keywords: Liver transplantation; Immune suppression; Target of rapamycin inhibitors; m-TOR; Sirolimus; Everolimus; Hepatocellular carcinoma; Hepatitis C virus.
Received 7 December 2010; received in revised form 28 June 2011; accepted 29 June 2011

*Corresponding author. Address: 2D4.44 Mackenzie Center, University of Alberta, Canada T6G 2B7.
E-mail address: norm.kneteman@albertahealthservices.ca (N.M. Kneteman).

Abbreviations: AFP, alpha fetoprotein; CNIs, calcineurin inhibitors; CKD, chronic kidney disease; CPM, central pontine myelinolysis; CsA, cycrosporine; EVRL, everolimus; FDA, Food and Drug Administration; GFR, glomerular filtration rate; HCC, hepatocellular carcinoma; HCV, hepatitis C virus; IS, immunosuppression; LTx, liver transplantation; TLR, Toll-like receptor; m-TORi, mammalian target of rapamycin inhibitors; Tregs, regulatory T cells; MMF, mycophenolate mofetil; SRL, sirolimus; Tac, tacrolims; VEGF, vascular endothelial growth factor.

identified as a potent antifungal metabolite. This macrolide, produced by *Streptomyces hygroscopicus*, inhibited cell proliferation and so produced antitumor and immunosuppressive activity [11]. In 1999, SRL was FDA approved for prevention of kidney allograft rejection [12]. Rapamycin and three analogs modified at C43 to increase solubility and bioavailability have undergone clinical evaluation. The addition of an ester, ether, or phosphonate group yield temsirolimus, EVRL, and deferolimus, respectively. SRL and EVRL are mainly used as immunosuppressive medications in transplantation and are the focus of this review (Fig. 1).

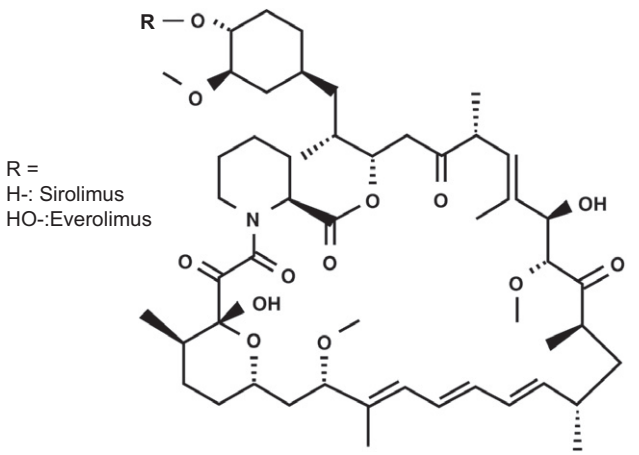

Fig. 1. Structures of sirolimus and everolimus.

The m-TOR signaling pathway

TOR was identified in yeast followed by the discovery of the m-TOR. m-TOR is a key signaling kinase that affects broad aspects of cellular functions, including metabolism, growth, survival, aging, synaptic plasticity, and memory. Rapamycin engages FK506-binding protein 1A, 12 kDa (FKBP12); the complex engages and inhibits TOR but not calcineurin, thereby blocking cell cycle progression at the G1 to S phase, causing inhibition of T cell proliferation [13].

As shown in Fig. 2, the m-TOR pathway is activated by a variety of different classes of stimulations. There are at least two distinct m-TOR complexes, m-TOR complex1 (m-TORC1) and m-TOR complex2 (m-TORC2), that have distinct relationships both to upstream and downstream effectors and to each other [14,15]. Signals from growth factors (insulin or IGF-1), various cytokines, co-stimulatory signals, Toll-like receptor (TLR) ligands, cellular energy levels, hypoxia, cellular stress and DNA damage determine m-TORC1 activity. These signals mediate their effects through the tuberous sclerosis complex 1 (TSC1)-TSC2 complex, which is the main negative regulator of m-TORC1. Activated m-TORC1 promotes mRNA translation by stimulating S6 kinase (S6K1) and inhibiting EIF4EBP1 (eukaryotic translation initiation factor-binding protein 1). m-TORC2 is not inhibited directly by rapamycin, although long-term rapamycin administration disrupts its assembly in some cells. m-TORC2 regulates actin cytoskeletal dynamics through the small GTPase RAS homologue (RHO) and protein kinase C (PKC).

The m-TOR in immunity and mechanism of immunosuppression

m-TORi: impact on innate immunity

In addition to the regulating effects of m-TOR in dividing cells, it has been recently demonstrated that m-TOR affects the innate immunity system [16]. Inhibition of m-TOR promotes pro-inflammatory cytokines such as IL-12 and IL-1β, inhibits the anti-inflammatory cytokines such as IL-10, and boosts MHC antigen presentation via autophagy in monocytes/macrophages and dendritic cells. Moreover, m-TOR regulates type1 interferon production and the expression of chemokine receptors and co-stimulatory molecules [17]. m-TORi blocks progression from G1 to S phase in natural killer (NK) cells but does not affect interferon-γ production in primary NK cell lines; cytotoxicity assays showed modestly decreased NK cell activity against the YAC-1 cell line [17]. *In vivo* study in a rat to hamster skin xenograft model did not show significant effects [17,18].

m-TORi: impact on adaptive immunity

Inhibition of innate immunity by m-TORi affects adaptive immunity via co-stimulatory molecules and cytokine production. m-TORi also marked thymic involution, which is associated with decreased T cell output [17]. By blocking cell cycle progression from G1 into S phase in IL-2 stimulated T cell lymphocytes [18]; rapamycin potently decreases the proliferation of $CD4^+$ T cells, although it does not alter the proportion of $CD4^+$ single positive T cells that upregulate their expression of forkhead box P3 (FOXP3) in the thymus [16,17]. Furthermore, m-TOR-deficient $CD4^+$ T cells efficiently differentiate into $FOXP3^+$ regulatory T cells (Tregs) upon stimulation, compared to wild-type. Differentiation into T-helper (Th) 1, Th2, or Th17 cells was severely inhibited in m-TOR-deficient $CD4^+$ T cells even in the presence of appropriate polarizing cytokines [19]. m-TORi may be permissive to induction of Tregs in organ transplantation [19], another potential mechanism for immune suppression. Detail of T cell mechanisms regulated by m-TOR have been reviewed [21].

Pharmacology of m-TORi

The half-life of SRL and EVRL is approximately 60 h and 30–40 h, respectively, and EVRL has a more rapid time to steady state (4 days versus 6 days for SRL) [20,21]. Both compounds are cleared through the liver via the hepatic cytochrome P450-3A4 microsomal system, which is the same metabolic pathway used by cyclosporine and tacrolimus (Tac). Drugs which inhibit or compete with the activity of cytochrome P450 system may significantly impair the clearance of both SRL and EVRL and lead to significant increase in systemic levels. Common drugs that may cause clinically significant elevations in blood concentrations through inhibition of metabolism include fluconazole, azithromycin, and protease inhibitors.

Clinical experience with m-TORi: adverse events and risks

A rationale for selective substitution of m-TORi for CNIs in LTx depends on evidence for effective immune suppression and a