Medical Aspects of mTOR Inhibition in Kidney Transplantation

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The advances in transplant immunosuppression have reduced substantially the incidence of kidney graft rejection. The focus has moved from preventing rejection to preventing the long-term consequences of long-standing immunosuppression, including nephrotoxicity induced by calcineurin inhibitors (CNI), as well as infectious and neoplastic complications. Since the appearance in the late 1990s of mTOR inhibitors (mTORi), these unmet needs in immunosuppression management could be addressed thanks to their benefits (reduced rate of viral infections and cancer).

Keywords: kidney ; transplant ; kidney transplant ; immunosuppression ; mTOR ; mTOR inhibition

1. Introduction

The landscape of kidney transplantation has changed notably, moving from an incidence of acute kidney graft rejection of >80% in the early ages to <10% nowadays, as a result of the advances in transplant immunosuppression. Initially, it was based on steroids and azathioprine (AZA), but the current gold standard, also recommended by the KDIGO guidelines, includes first-line induction therapy with basiliximab in association with a calcineurin inhibitor (CNI) (preferably, tacrolimus, TAC) and an antiproliferative agent (preferably, mycophenolate), with the possibility of early steroid withdrawal in low-risk recipients. In recipients with a high immunological risk, the suggested first-line induction therapy is represented instead by lymphocyte-depleting agents [1]. Moreover, in recent years the focus has moved from preventing rejection to preventing the long-term consequences of long-standing immunosuppression. Among them, nephrotoxicity induced by CNI and infectious and neoplastic complications have to be highlighted.

2. Pharmacology of mTOR Inhibitors

In 1964,on the South Pacific island of Rapa Nui (Easter Island), a Canadian expedition took soil samples, aiming to discover novel antimicrobial agents. Later, it was discovered that one of the compounds extracted by Streptomyces hygroscopicus had immunosuppressive, antitumor and antifungal activity [2][3][4]. These properties were due to the interaction of the molecule with an immunophillin (FKBP-12) that was necessary to inhibit cell growth and proliferation [5]. Curiously, the same immunophillin mediates signal transduction for TAC [6]. This substance was named Rapamycin (RAPA) on behalf of the name of the island and clinically is known as sirolimus (SRL).

In the following years, different groups discovered that the target of RAPA was a multiprotein complex analog to the yeast TOR gene [7][8][9][10], so it was named as mechanistic (formerly mammalian) Target of Rapamycin (mTOR) [10]. Further discoveries established that mTOR is a serine/threonine protein kinase that forms the catalytic subunit of the two largest multiproteic complexes, mTOR Complex 1 (mTORC1) and mTOR Complex 2 (mTORC2) (**Figure 1**) [11]. Recently, mEAK-7 (mTOR associated protein, eak-7 homolog) was identified as a positive activator of mTOR signaling via an alternative mTOR complex and it has been theorized that this novel complex is a third member of known mTOR complexes, mTORC3 [12][13].

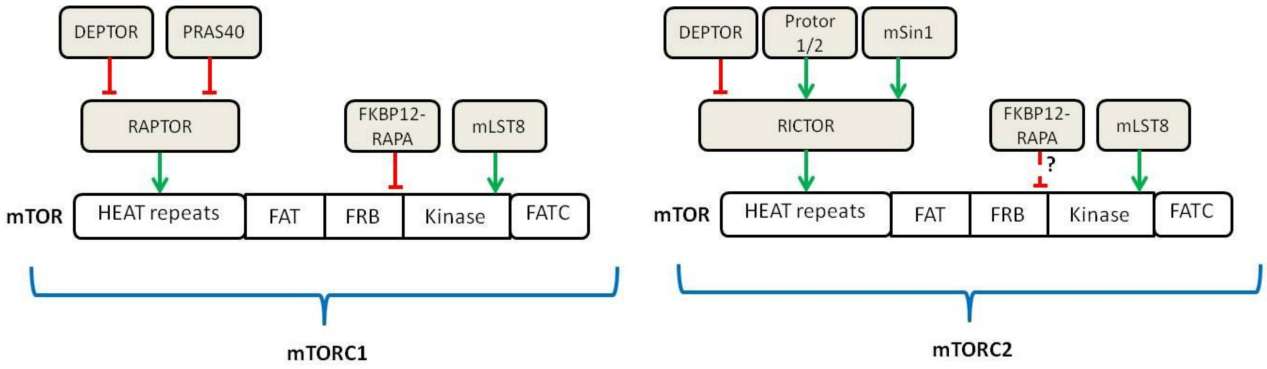

Figure 1. Schematic of the components belonging to mTORC1 and mTORC2 [11]. Green lines show activating signals, red lines show inhibitory signals, dashed lines indicate that the exact mechanism is unknown.

The key components associated with mTOR in mTORC1 are RAPTOR (Regulatory Associated Protein of mTOR) and mLST8. In turn, two inhibitory components of the complex are PRAS40 (Proline-Rich Akt Substrate of 40 kDa) and DEPTOR (DEP domain-containing mTOR-interacting protein) [14][15]. The components participating in the mTORC2 complex include mLST8, DEPTOR and RICTOR (Raptor-Independent Companion of mTOR), with its related regulatory proteins, mSin1 and Protor 1/2 [16][17][18].

In contrast to mTORC1, mTORC2 is not affected by acute treatment with RAPA. However, chronic RAPA treatment inhibits mTORC2 signaling; this seems to be due to the incapacity of RAPA-bound mTOR to incorporate into the newly assembled mTORC2 complexes [19]. Initially, it was thought that mTOR complex was cytosolic; later on, it became clear that upon activation, mTORC1 localizes at the surface of lysosomes in a process that is mediated by cytoplasmatic nutrients, especially amino acids [20].

3. Use of mTOR Inhibitors in Graft-versus-Host Disease

Rapamycin and its analogs have been increasingly used to prevent graft-versus host disease (GVHD) after bone marrow transplantation (BMT). GVHD still represents the major complication after BMT, resulting in life-threatening complications for the recipient. It occurs when T cells in the transplant become activated by alloantigens and subsequently destroy recipient tissues [21][22].

Whilst promising response rates particularly for the treatment of chronic GVHD have been reported, the toxicity profile particularly in combination with CNIs remains limiting. Also, they have been used for GVHD prevention as it has been shown that RAPA treatment can induce the accumulation of regulatory T cells (Treg) in the skin of mice after bone marrow transplantation. In a recent study, Scheurer et al. found that RAPA treatment can increase the immunosuppressive potential of myeloid-derived suppressor cells (MDSCs) whilst maintaining the anti-tumor cytotoxicity of T cells (graft vs. tumor) without impairing the induction of Treg in a bone marrow transplantation mouse model. However, other in vitro studies and clinical findings demonstrated that the development of RAPA resistance typically occurs [21][22].

Thus, future use of mTOR inhibitors may rather favour prophylaxis than treatment of GVHD. Here, combinations without CNIs may offer promising prophylactic regimens with low toxicity rates [22]

4. Use of mTOR Inhibitors in Kidney Transplantation

The current state of the art with mTORi is the quest to discover the optimal immunosuppressive schedule that could guarantee kidney transplant recipients the lowest incidence of rejection and the best safety and long-term renal function. Thanks to all the basic, translational and clinical research achieved in the last twenty years, mTORi is now used as de novo immunosuppression in association with CNI at trough levels of 3–8 ng/mL. Another possibility is represented by the conversion of either CNI or mycophenolate (MPA) to an mTORi later on after transplantation. This can be beneficial in cases in which CNI- or MPA-related toxicity are evident, such as nephrotoxicity, tremor, leucopenia, diarrhea or CMV replication, which warrant a change in the immunosuppressive schedule. In these cases, late conversion can be carried out safely for most patients, especially from MPA to mTORi.

Moreover, different combinations of mTORi with the other immunosuppressive drugs have been investigated. Due to the narrow therapeutic index and the vast effects induced by mTORC1 and mTORC2 on human health and metabolism, management of side effects was challenging and hands-on experience was needed. Initially, it was not even clear that checking the trough level was necessary [23], as some trials focused only on the oral dose and not on therapeutic drug

monitoring [24]. The general feeling about mTORi in the transplant community fluctuated from enthusiasm to disappointment, and vice-versa, given the brilliant discoveries and the frustrating failures. As a matter of fact, what is known about mTORi in kidney transplantation derives from the sum of pre-clinical and clinical data that have highlighted the strengths and the weaknesses of mTORi in this setting.

5. Real-Life Use of mTOR Inhibitors in Renal Transplantation

All the lessons learned by all these randomized clinical trials taught the transplant community how to take advantage of the benefits of mTORi (reduced rate of viral infections and cancer), without paying an excessive price for their side effects. It is also important to bear in mind the strict inclusion criteria of the TRANSFORM trial. Patients at high immunological risk were discarded, as well as recipients of a Donors after Circulatory Death (DCD), which represent a valuable source of donors in many countries. In this field, a single-center propensity score analysis published in 2020 by the researchers' group [25] verified the real-life feasibility of using a TAC–mTORi combination scheme through 401 patients that were analyzed according to the baseline immunosuppression (TAC associated with either MPA or mTORi). mTORi were administered irrespective of the type of donor (non-heart beating or not) and the immunological risk of the recipient. Patients that would have not entered the TRANSFORM trial for these and other exclusion criteria accounted for 52.9% of the total population. Curiously, patients who met the TRANSFORM inclusion criteria (n = 186) had very similar results to that of the original trial, with no differences in terms of 1-year and last follow-up graft rejection and survival between the MPA and mTORi group. On the other hand, patients that could not have participated in the trial (n = 215), had better results for both outcomes. Another strong point in favor of mTORi was the evidence in all groups of better 1-year and last follow-up patient survival. A reduced rate of infection-related hospitalizations during the first year could partially justify this finding. On the other side, a higher incidence of drug discontinuation was observed in the mTORi group due to classical side effects, including hypercholesterolemia, proteinuria, surgical-associated complications, etc., as well as beneficial effects (reduced CMV reactivation and total number of infections requiring hospitalization).

A difference worthy to mention with respect to the TRANSFORM trial was the higher trough levels of TAC in patients treated with mTORi; this may also justify the decreased incidence of rejection in this group. This different attitude about TAC/mTORi trough levels was not associated with a worse 1-year renal function and higher chronicity scores at protocol renal biopsy [25].

In a sub-analysis of the same population focused on high immunological risk patients, defined as a baseline cPRA ≥ 50% (n = 71), the combination TAC + mTORi was associated with better results in terms of 1-year rejection-free survival compared to TAC + MPA (incidence of biopsy-proven acute rejection was 15.2% versus 36.8%, respectively) [26]. This striking difference in results in comparison with the US92 trial [27] is probably attributed to the higher TAC trough levels employed [25][26]. This probably indicatesthat in the high immunological risk population, TAC should not be minimized as in the low-risk population studied in the TRANSFORM trial.

6. Practical Use of mTOR Inhibitors in Kidney Transplantation—Troubleshooting

The two mTOR inhibitors commercially available and approved for use in kidney transplantation can be started soon after surgical intervention at a dose of 1–2 mg qd (Sirolimus, SRL) or 1–1.5 mg bid (Everolimus, EVL), with the aim to reach trough levels of 3–8 ng/mL. During the first weeks after kidney transplant, it is advisable, however, to maintain trough levels in the range of 3–5 ng/mL.

In the researchers' center, SRL and EVL are associated with TAC in order to reach a sum (TAC + mTORi) of trough levels of 8–12 ng/mL [25][26]. This sum can be reduced to 8–10 ng/mL at 6–12 months after kidney transplantation, according to individual assessment of rejection risk. Particularly, TAC can be minimized to <5 ng/mL after 6–12 months in the low-risk population according to the TRANSFORM experience [28]. In patients with high immunological risk, it is advisable not to minimize TAC during the first year after kidney transplantation and to consider reducing trough levels thereafter, according to local center policies and, preferably, to the results of per-indication or per-protocol kidney graft biopsies.

Induction should be based on individual risk assessment depending on the immunological risk (i.e., anti-CD25 antibodies for low-risk patients and anti-thymocyte globulins for the high-risk population).

Contraindications for the start of de novo mTOR inhibitors in kidney transplantation include: a previous history of intolerance or side effects with mTORi, chronic obstructive pulmonary disease, central obesity that could impair surgical wound healing, thrombotic microangiopathy as the cause of end-stage renal disease, and any patient at risk of surgical

complications and possibly re-intervention. Patients that could benefit most from the use of mTOR inhibitors are those with a history of virally induced cancers and who are at risk of developing CMV disease or BK nephropathy.

Advantages for the use of mTOR inhibitors in comparison with MPA include, undoubtedly, less incidence of viral infections (especially, CMV and BK), less neutropenia and low blood platelets, and a possible reduction in long-term incidence of solid neoplasia, especially for non-melanoma skin cancer in which the evidence is more convincing [29][30]. Moreover, in low immunological risk patients, mTORi could allow safe minimization of CNI, which in the long term could theoretically prolong graft survival.

The most common side effects associated with the use of mTOR inhibitors are listed in **Table 1**, along with a list of possible solutions.

Table 1. Most common side effects of mTOR inhibitors in kidney transplantation with a list of possible solutions.

Side Effect	Solution
Neumonitis	Discontinue mTORi.
Thrombotic microangiopathy	If clinically evident and in case of rejection, consider discontinuing mTORi. If it is only a finding in renal biopsy without clinical deterioration, consider reducing trough levels of either CNI or mTORi or both. In low-risk patients consider conversion from CNI to MPA.
Surgical scar infection or late healing	Switch to MPA until resolved and then switch back to mTORi.
Lymphocele	Switch to MPA until resolved and then switch back to mTORi.
Productive surgical drainage	Switch to MPA until resolved and then switch back to mTORi.
Post-transplant diabetes mellitus	Start of oral antidiabetic agent and/or insulin. Consider switching TAC to CsA.
Hypertriglicerydemia	Diet, weight loss, omega-3 fish oil.
Hypercolesterolemia	Diet, weight loss, statins, ezetimibe, fibrates.
Proteinuria	Consider using ACE inhibitors or Angiotensin Receptor Blockers.
Edemas	Consider using diuretics. In patients taking vasodilators (such as amlodipine), consider switching to another anti-hypertensive agent.

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