Abstract

Acute rejection episodes are now as low as 5–20% in the first year after renal transplantation; however, graft half-life has remained almost unchanged in the last decade. This statistic is mainly attributable to the side effects of immunosuppression, with loss of allografts due to the chronic allograft nephropathy that is a consequence of calcineurin inhibitor toxicity or hypertension. Patient death due to cardiovascular events, infections and malignancy also contribute to allograft loss. The introduction of the inhibitors of the mammalian target of rapamycin sirolimus and everolimus in renal transplantation has increased the repertoire of immunosuppressive protocols substantially. They have a different mode of action and a different side effect profile (i.e. lower nephrotoxicity, less hypertension and less neoplastic potential) than the calcineurin inhibitors. The inhibitors of the mammalian target of rapamycin therefore provide an especially promising alternative for the maintenance immunosuppression after renal transplantation. This overview provides a summary of the current literature on inhibitors of the mammalian target of rapamycin, with a special focus on sirolimus.

Keywords: everolimus; mTOR; renal; sirolimus; transplantation

Introduction

With the newer immunosuppressive protocols, acute rejection episodes in the first year after renal transplantation are as low as 5–20%, and graft loss due to acute rejection is rare. Biopsy-confirmed acute rejection (BPAR) is the primary end-point in multiple transplantation studies. However, as we learned from databases and large transplantation trials, there is only a mild association between an early acute rejection episode and graft survival. Therefore, despite reduced acute rejection episodes, average graft half-life following renal transplantation has remained almost unchanged in the last decade [1]. In recent years, concern has shifted to factors influencing long-term allograft and patient survival, patient health and quality of life. Although chronic allograft nephropathy (CAN), characterised by interstitial fibrosis and tubular atrophy, is the main cause of renal allograft loss, death from cardiovascular and infectious complications or malignancy in patients with a functioning allograft account for about 50% of graft losses. With the utilisation of more potent immunosuppressive agents, the incidence of de novo malignancies has increased [2]. The direct oncogenic effects of the immunosuppressive agents are at least partly responsible for this increased rate [3]. Therefore, long-term patient and allograft survival can only be improved by a reduction of the side effects of immunosuppressive agents. Some of the more adverse effects, such as arterial hypertension, nephrotoxicity, diabetes mellitus, neurotoxicity, and de novo malignancies, have a deleterious impact on quality of life and patient survival. In the last two decades, there has been much effort to reduce these side effects by employing new combination therapies or by finding and adjusting optimal regimens, dosages and blood levels. Meanwhile, there was a substantial need to find immunosuppressive regimens with less neoplastic potential at comparable immunosuppressive activity. The reported antifungal, antitumoral and immunosuppressive activity of inhibitors of the mammalian target of rapamycin (mTOR) and rising expectations for immunosuppressive therapy without nephrotoxicity attracted special attention to this new class of immunosuppressive agents [4]. In recent years, sirolimus (SRL) and everolimus (RAD), the first mTOR inhibitors, have been implemented in many clinical trials with respect to their risk-benefit assessment. This review summarises the relevant literature on SRL in renal transplantation.

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Inhibitors of the mammalian target of rapamycin

SRL (Rapamycin, Rapamune®), a lipophilic microcyclic lactone, was isolated from a strain of fungus called Streptomyces hygroscopicus discovered at Rapa Nui (Easter Island). SRL was found to have potent immunosuppressive activity with a distinct mechanism of action vis-à-vis other immunosuppressants. It structurally resembles tacrolimus (Tac) and binds the FK binding protein 12 (FKBP-12). In this way, it forms an immunophilin complex that serves as a catalyst. The target of the SRL-FKBP-12 complex is the serine-threonine kinase of the phosphatidylinositol-3-kinase pathway, which is called mTOR and acts during co-stimulatory and cytokine-driven pathways. mTOR has been identified as the principal controller of cell growth and proliferation. The SRL-FKBP-12 complex inhibits mTOR-mediated signal transduction pathways by blocking post-receptor immune responses to co-stimulatory signal 2 during G0 to G1 transition and to cytokine signal 3 during G1 progression. It also inhibits the IL-2- and IL-4-dependent proliferation of T- and B-cells leading to suppression of new ribosomal protein synthesis and arrest of the G1-S phase of the cell cycle [4–7]. Proliferation of non-immune cells, such as fibroblasts, endothelial cells, hepatocytes and smooth muscles is also impaired by inhibition of the growth-factor-mediated responses (i.e., basic fibroblast growth factor, platelet-derived growth factor, vascular endothelial cell growth factor and transforming growth factor-β) [8]. Additionally, it has been shown that mTOR takes part in several protein synthesis pathways that could be involved in oncogenesis.

More recently, RAD (Certican®, Basel, Switzerland), the 40-O-(2-hydroxyethyl)-derivative of SRL, was developed. Like SRL, RAD blocks growth-factor-stimulated cell proliferation of haematopoietic and non-haematopoietic cells by forming a complex with the intracellular immunophilin FKBP-12. The affinity of RAD for FKBP-12 is lower than that of SRL; 50% inhibition is achieved at a 3-fold higher dose [9].

Sirolimus in renal transplantation

The promising results of the phase I clinical trial of SRL by Kahan et al. led to the first randomised, placebo-controlled, multi-centre phase II clinical trial [10]. This study evaluated the combination of SRL with cyclosporine A (CsA) and steroids when compared with a placebo, CsA and steroids for the prophylaxis of acute renal allograft rejection [11]. This multi-centre study demonstrated that the incidence of BPAR within the first six months after renal transplantation was significantly reduced in the full-dose CsA group receiving 1 or 3 mg/m²/d SRL compared with the control group. Moreover, reduced-dose CsA with 1 mg SRL resulted in significantly better renal function, indicating that co-administration of SRL might permit CsA dose reduction without jeopardising organ function.

Encouraged by these results, two large phase III multi-centre trials enrolling nearly 1300 renal transplant recipients were performed in the US and Europe. In the American study, 719 renal allograft recipients were randomly assigned to receive 2 mg/d SRL, 5 mg/d SRL or azathioprine (AZA) [12]. The rate of efficacy failure at six months, e.g. BPAR, graft loss or death, was significantly lower in the two SRL groups compared to the AZA group. One-year graft and patient survival, infection and malignancy were similar in all groups. In the European trial, 576 renal allograft recipients were randomised to receive 2 or 5 mg/d SRL or a placebo in combination with CsA and steroids in all groups [13]. Efficacy failure and BPAR at six months were significantly lower in the SRL groups than in the placebo group. The successful results of SRL in the phase II and III clinical trials led to the first FDA approval of SRL in combination with CsA and steroids in kidney transplantation in 1999. In the meantime, SRL was evaluated in CsA-sparing protocols. In 11 European centres, renal allograft recipients (n = 83) were randomised to receive either CsA or SRL, with administration of steroids and AZA in both groups [14]. One-year results showed similar graft and patient survival rates and a comparable incidence of BPAR. Serum creatinine was significantly lower in the SRL group; however, side effects were more frequent with SRL administration. In a second clinical trial, performed in 14 European centres, renal allograft recipients (n = 78) were randomised to receive either SRL or CsA, with administration of steroids and mycophenolate mofetil (MMF) in both groups [15]. Graft and patient survival as well as BPAR were similar at one year. SRL-treated patients had better renal function with a higher calculated glomerular filtration rate (GFR). The results of the two above-mentioned trials were pooled by Morales et al. In this secondary analysis, renal function after 24 months was significantly better in the SRL group compared to the CsA group [16]. The favorable results of previous trials led to further studies on the efficacy of CsA withdrawal from SRL-based immunosuppressive regimens. Johnson et al. assessed whether CsA elimination from a SRL-CsA-steroid regimen at month 3 is safe [17]. In this study, from Europe, Australia and Canada, 525 renal allograft recipients received 2 mg SRL, CsA and steroids. By month 3, patients were divided into two groups: one remained on the SRL-CsA-steroid regimen and the other had CsA withdrawn, with therapy continued at a higher dose of SRL. The results showed no difference in graft or patient survival, whereas the acute rejection rate was significantly higher for the SRL-steroid group. However, renal function measured by GFR and blood pressure was ameliorated when CsA was withdrawn. The authors concluded that SRL could be applied as maintenance therapy with avoidance of long-term side effects of CsA administration such as nephrotoxicity. On the other hand, this study as well as previous trials demonstrated that SRL may also potentiate the nephrotoxicity of CsA when it is applied...
Sirolimus in renal transplantation

There are no distinct guidelines for conversion to SRL in renal transplantation, however, transplantation experts in Germany defined the following possible indications for a conversion to SRL-based immunosuppression (Arns W and Diekmann F, Consensus statement): CNI-associated side effects such as nephrotoxicity, deterioration of renal function, arterial hypertension and diabetes mellitus; chronic transplant dysfunction, “creeping creatinine”; possibility of tumor occurrence or progression with the administration of a CNI. The contraindications of conversion to SRL-based therapy are serum cholesterol of >300 mg/dl and/or serum triglycerides >400 mg/dl, despite lipid-lowering agents before conversion; advanced renal insufficiency with a serum creatinine level exceeding 4 mg/dl; glomerular damage with proteinuria >1 g/day. The administration of SRL in high-risk patients, such as those with a body mass index >30 kg/m², diabetes mellitus, major re-operations or delayed graft function, should not take place until four to six weeks after renal transplantation, when the wound healing process is nearly completed and the serum creatinine should be under 2.5 mg/dl. The dosage of MMF or AZA should be reduced by 50% at the time of SRL administration while steroid therapy should be regularly continued. The starting dose of SRL corresponds to the maintenance dose of 4–6 mg/d, and the dose of the CNI must simultaneously be reduced by 50%. When the SRL trough level is reached at 5–10 ng/mL, the CNI can be stopped completely. Further trough level measurements are recommended weekly after each additional dose change as well as up to the point when desired trough levels have been reached. Extra trough level measurements are indicated in patients with liver malfunction or when using specific substances that might inhibit CYP3A4 and/or P-gp metabolism. In the meantime, first solid data on conversion from a CNI to a SRL-containing regimen have emerged. The recently presented CONVERT study enrolled 830 renal transplant recipients on CNI (either CyA or Tac) therapy. Patients were randomly assigned to either continue the CNI (n=275) or to undergo conversion from the CNI to SRL (n=555). At 12 months, significantly more of the patients treated with SRL vis-à-vis CNI demonstrated improvements in GFR. However, this was no longer applicable for patients with a high protein-to-creatinine ratio prior to conversion [24].

Antitumoral activity of sirolimus

Regarding the antitumoral activity of SRL, it has been shown that SRL inhibits the proliferation of transformed cell lines of lymphoid, CNS, hepatic, melanocytic, osteoblastic, myogenic, renal and connective tissue origin as well as proliferation of T and B cells transformed by HTLV-1 and EBV [4]. In experimental studies, SRL exhibits antiproliferative effects on...
certain types of tumors, including renal cell cancer, rhabdomyosarcoma, B- and T-cell lymphoma, glioma, breast cancer, colon carcinoma, multiple myeloma, ovarian cancer, prostate cancer, bladder cell carcinoma, non-small cell lung cancer, pancreatic cancer, melanoma and hepatocellular carcinoma. SRL also has antiangiogenic capabilities that could prevent tumor growth [8]. Several clinical trials are under way or have been carried out on a variety of cancers. The preliminary results of 5 multi-centre studies regarding the incidence of skin cancers, are especially promising [25]. These multi-centre studies comprised a total of 1886 patients. The incidence of skin malignancy two years after transplantation was lower in patients treated with SRL, especially in those who had CyA withdrawn. In another study, Luan et al. demonstrated that SRL could change tumor cells from invasive phenotypes like spindle- or dome-shaped cells to a less invasive cuboidal shape in renal cell carcinoma [26]. In the CONVERT trial mentioned earlier, the incidence of post-transplant malignancy in patients on SRL therapy was significantly lower compared to patients who remained on a CNI (3% vs 10%) [24]. Some SRL analogues are now tested as pure antitumoral agents in phase I and II trials.

Side effects of sirolimus

The mTOR inhibitors have considerable side effects that are sometimes limiting to therapy. About 30–50% of patients on SRL therapy go off it during follow-up due to the related side effects. Side effects include increased serum lipids, decreased hemoglobin, arthralgia, peripheral edema, gastrointestinal complaints, skin disorders, stomatitis, electrolyte disturbances (e.g. hypokalemia and hypophosphatemia), dyspnea, cough, infectious diseases and a higher incidence of lymphoceles [27]. One of the more serious side effects is the development of SRL-induced interstitial pneumonitis. This entity is especially associated with high SRL dose or drug levels [28]. Patient deaths related to SRL-induced pneumonitis have been reported in heart transplant recipients.

Off note, mTOR inhibitors are often classified as nonnephrotoxic. We know from experimental as well as patient studies, however, that mTOR inhibitors affect renal structure and function. The mTOR inhibitors impaired recovery from ischaemia/reperfusion injury in animal models of renal transplantation [29]. When given with CNI, mTOR inhibitors worsen CNI toxicity, and this is not only the effect of higher CNI drug levels. Delayed graft function is also more frequent with the use of mTOR inhibitors than with other drug classes. This is most likely due to an increased rate of acute tubular necrosis. Most importantly, there is now solid evidence that switching to SRL from a CNI might promote de novo allograft proteinuria. Histological lesions in biopsied patients resemble focal segmental glomerulosclerosis; however, it is not clear whether this is recurrent primary kidney disease, chronic allograft damage or the consequence of CNI withdrawal and/or SRL exposure [30]. Other authors report a tubular dysfunction with SRL therapy, which is responsible for the increase in proteinuria.

Conclusions

The introduction of mTOR inhibitors in renal transplantation has increased the repertoire of immunosuppressive protocols substantially. The mTOR inhibitors SRL (as well as RAD) have proven their efficacy and safety in numerous studies and are used either de novo or as a substitute in the follow-up after renal transplantation. The mTOR inhibitors have a different mode of action and a different side effect profile than the CNIs. Wound healing disturbances and lymphoceles as well as haematological side effects are seen more often with mTOR inhibitors, whereas nephrotoxicity, hypertension and malignancies are observed frequently with CNIs. The various advantages and disadvantages of CNIs and mTOR inhibitors at different points in time after renal transplantation led to the concept of sequential immunosuppression. Today, many patients receive a CNI during the early postoperative period, when acute rejection episodes and wound healing disturbances occur. In the long run, however, the CNI may be replaced by an mTOR inhibitor to reduce nephrotoxicity and the occurrence of de novo malignancy. When tolerated by the patient, there is a clear preference for mTOR inhibitors as a maintenance immunosuppressant after renal transplantation. However, side effects are frequent, and up to 50% of patients eventually have to discontinue the mTOR inhibitor.

Conflict of interest statement. None declared.

Reference

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24. Univariate and multivariate analyses of factors affecting renal allograft function after conversion from calcineurin inhibitor (ci)- to sirolimus (srl)-based immunosuppression: results from the multicenter convert trial. *Transplantation* 2006; 82: 412


