The role of proliferation signal inhibitors in post-transplant malignancies

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Abstract

The proliferation signal inhibitors (PSIs; mammalian target of rapamycin (mTOR) inhibitors) are widely used for immunosuppression in transplant recipients. Alongside their immunosuppressive properties, PSIs also have substantial anti-neoplastic activity, as a result of their inhibition of cellular signalling pathways involved in critical functions such as cell division, T-cell activation, invasion and growth factor production. In vitro and in vivo studies have shown that PSIs can prevent the growth of experimentally transformed cells and tumour-derived cell lines, and can also increase the sensitivity of cells to apoptosis-inducing agents. The mechanisms of anti-tumour activities of PSIs identified in pre-clinical studies include up-regulation of adhesion molecules with reversion to less invasive phenotypes, and inhibition of angiogenesis resulting from both decreased vascular endothelial growth factor production and decreased endothelial sensitivity to such growth factors. In clinical trials of PSIs in transplant recipients, results show that the incidence of malignancies is substantially lower in patients receiving PSIs than in those receiving calcineurin inhibitor (CNI)-based immunosuppression, with significantly longer times to the development of malignancy. In clinical trials of PSIs in transplant recipients, results show that the incidence of malignancies is substantially lower in patients receiving PSIs than in those receiving calcineurin inhibitor (CNI)-based immunosuppression, with significantly longer times to the development of malignancy. This protective effect of the PSIs is also present in patients receiving combination therapy with a CNI and a PSI. There is also evidence to suggest a role for PSIs in the management of post-transplantation malignancy, with reports of complete resolution of primary and metastatic tumours after conversion from a CNI to a PSI. These beneficial effects have led to the investigation of everolimus and an analogue of sirolimus as a treatment for patients with advanced solid tumours.

Keywords: cancer; everolimus; proliferation signal inhibitors/mTOR inhibitors; sirolimus; transplantation

Introduction

In addition to their potent immunosuppressive properties, the proliferation signal inhibitors (PSIs; also known as mammalian target of rapamycin (mTOR) inhibitors) everolimus and sirolimus have been shown to have substantial anti-cancer activities. An analogue of sirolimus, CCI-779, has been developed as an anti-neoplastic agent.

PSIs inhibit mTOR, which is a critical signalling pathway for several growth factors [1]. Phosphatidylinositol-3 kinase (PI3K) is activated by receptor binding by growth factors and cytokines such as interleukin 2 and vascular endothelial growth factor (VEGF), leading to phosphorylation of Akt and activation of mTOR [1]. The pathway is regulated by phosphatase and tensin homologue deleted on chromosome 10 (PTEN), which is lost or mutated in many cancers, leading to increased activation of mTOR, thus making the cells resistant to apoptosis.

Inhibition of the mTOR protein leads to a 15–20% reduction in overall protein synthesis, leading to apoptosis, inhibition of T-cell activation, reduced cell migration and invasion, structural changes, autophagy and decreased expression of growth factors such as VEGF (Figure 1) [1–3]. Angiogenesis and the ability to inhibit apoptosis are necessary processes for the development and propagation of malignant cells. Disruption of these processes can potentially be achieved by the inactivation of mTOR. These molecular pathways have been shown to be of practical importance for transplant patients, as demonstrated both in pre-clinical and clinical studies.

Proliferation signal inhibitors and post-transplant malignancies: pre-clinical data

The role of PTEN in the anti-neoplastic effects of PSIs has been explored in vitro and in vivo using mouse and human cell lines. A study using the analogue CCI-779 (temsirolimus) showed that growth of knockout mouse cells negative for PTEN and human tumour cell lines with no PTEN expression could be blocked [4].
Fig. 1. The phosphatidylinositol-3 kinase signalling pathway downstream of mTOR, showing how mTOR inhibition can prevent cellular activities relating to (a) the cell cycle and apoptosis, and (b) adaptive responses, which are necessary for malignancy to develop [1] (Reprinted with permission from Elsevier). CDK, cyclin dependent kinase; 4E-BP1, eukaryotic translation initiation factor 4E binding protein 1; eIF4B, eukaryotic translation initiation factor 4B; eIF4E, eukaryotic translation initiation factor 4E; FKBP-12 rapamycin-associated protein [mTOR]; HIF1α hypoxia-induced factor 1α; mTOR, mammalian target of rapamycin; p70 S6k, p70 ribosomal protein S6 kinase; pRb, retinoblastoma protein; STAT3, signal transducer and activator of transcription 3; VEGF, vascular endothelial growth factor; VHL, von Hippel-Lindau.
Moreover, enhanced tumour growth caused by activation of Akt in PTEN-positive cells was also inhibited by CCI-779, providing further confirmation of the activity of PSIs downstream of the PI3K pathway. In studies with myeloma cells, survival of which requires activation of the PI3K pathway [2], Shi et al. [5] demonstrated that loss of PTEN function and constitutive activation of Akt led to a high level of sensitivity to CCI-779 that was more than 1000 times that of cells with functioning PTEN. Interestingly, activation of Akt in PSI-resistant myeloma cells with functioning PTEN did not convert them to a PSI-sensitive phenotype, and replacement of PTEN in a PSI-sensitive cell line did not confer resistance, showing that PTEN status alone does not determine sensitivity or resistance to the anti-neoplastic effects of PSIs.

In vitro, everolimus increases the sensitivity of tumour cells to DNA-damaging agents such as cisplatin in cells that have not lost the function of the tumour-suppressor gene p53 [6]. This effect is mediated by mTOR and is related to inhibition of p21, a target of p53 that is responsible for halting the cell cycle during DNA repair, preventing apoptosis of the damaged cell. In the absence of p21, the cells become more sensitive to the pro-apoptotic effects of cisplatin [6]. Further studies show that everolimus inhibits cell cycle progression. Cells derived from pulmonary post-transplant lymphoproliferative disorder (PTLD) from a liver transplant recipient were inhibited by everolimus, and an increased rate of apoptosis was demonstrated [7]. In vivo experiments from the same study demonstrated that everolimus, at a dose of 5mg/kg/day, inhibited tumour growth 10-fold compared with that in untreated severe combined immunodeficient (SCID) mice [7].

Similar data have been observed in in vivo models with both everolimus and sirolimus. Majewski et al. [8] have examined the effect of everolimus on Epstein–Barr virus (EBV)-transformed B-lymphocyte cell lines to provide a model of PTLD. Everolimus prevented cell division by halting the cell cycle in the G0/G1 phase and increased the rate of apoptosis in the transformed cells. When three of the cell lines were transplanted into SCID mice, everolimus again had a potent inhibitory effect on cell growth [8]. Furthermore, Majumder et al. [9], showed that prostate tumours induced in mice by expression of the human Akt1 protein can be reversed through mTOR inhibition. Luan et al. [10] examined the effect of sirolimus on a spontaneously arising renal cell tumour in mice, as well as on transplanted renal cell and transitional cell carcinoma cells in SCID mice. In the spontaneous renal cancer cells, sirolimus up-regulated expression of the adhesion molecule E-cadherin, inducing a change in phenotype from invasive spindle-shaped cells to non-invasive cuboidal cells that formed cell-cell attachments. Sirolimus also increased expression of p27 and reduced cyclin D1 levels, halting cell growth at the G1/S cell cycle checkpoint. In vivo, these molecular changes led to prevention of tumour growth and metastatic progress, and increased survival [10].

Sirolimus has been shown not only to decrease VEGF expression, but also to inhibit the response of vascular endothelial cells to VEGF [11]. The overall effect of these actions is to inhibit tumour angiogenesis, leading to a reduction in the growth of both metastases and established tumours in mice receiving normal immunosuppressive doses of sirolimus [11]. Reduction in tumour size has also been observed after everolimus treatment in a rat pancreatic tumour model [12]. Bodyweight was maintained, and everolimus was well-tolerated, with an anti-tumour activity similar to that seen with 5-fluorouracil (Figure 2). Notably, the effect of everolimus was dose dependent, and the efficacy of intermittent use suggests that it may be possible to achieve anti-neoplastic effects without inducing immunosuppression.

Fig. 2. Everolimus reduces tumour volume (A) while maintaining body weight (B) in a rat pancreatic tumour model [12] (Reprinted with permission from the American Association for Cancer Research).
Proliferation signal inhibitors and post-transplant malignancies: clinical data

In clinical trials, use of sirolimus has been associated with a reduced incidence of malignancies, particularly in patients converted from a calcineurin inhibitor (CNI) such as ciclosporin (CsA). In a pooled analysis of five sirolimus studies, the incidence of malignancy after 2 years was compared in patients receiving sirolimus with or without CsA [13]. After 2 years, results from two studies, in which 1295 patients were randomized to receive continuous CsA with sirolimus, azathioprine or placebo, showed that CsA + sirolimus was associated with a significantly lower incidence of skin cancer than was CsA + placebo. Moreover, two studies in which sirolimus base therapy was compared with CsA (n = 161) showed that 5% of the CsA-treated patients experienced some form of malignancy after 2 years, compared with none of those who received sirolimus. In the fifth study in this multi-centre report, in which CsA was eliminated from a regimen of CsA + concentration-controlled sirolimus, the incidence of malignancy was significantly lower in the elimination group (n = 215) compared with those who remained on CsA (n = 215) [13]. More recently, Campistol et al. [14] have reported the 5-year outcomes of this study. Survival analysis was used to determine the times to the development of a first malignancy, and to the development of a skin malignancy. After 5 years of follow-up, the median time to first skin carcinoma was significantly shorter in the CsA group (491 days) compared with the withdrawal group (1126 days; P = 0.07), with a relative risk of 0.346 in the CsA withdrawal group (P < 0.001) (Figure 3). The risk of non-skin cancer was also significantly reduced in the withdrawal group (4.0%) compared with the CsA group (9.6%; P = 0.032) [14]. A study of 158 renal transplant recipients receiving either sirolimus alone or in combination with CsA by Morales and colleagues [15] reported no malignancies in patients receiving sirolimus alone compared with 9.2% in those receiving sirolimus and CsA (P < 0.03). The beneficial effects of everolimus and sirolimus compared with the CNIs on post-transplant malignancy have also been reported in an analysis of 33249 patients from the Organ Procurement and Transplantation Network/United Network for Organ Sharing registry [16]. The results showed that the risk of any de novo malignancy was significantly lower in patients who received a PSI only (0.60%; P < 0.0001) than in those who received a CNI only (1.81%). Notably, the incidence of malignancy in patients who received combination therapy with a CNI and PSI was the same as that in those who received a PSI only. When de novo solid tumours were considered separately, 1.00% of patients receiving a CNI developed some form of solid tumour, compared with no patient who received a PSI only. Overall, the relative risks for the PSI group compared with the CNI group were 0.39 (P = 0.0002) for any malignancy and 0.44 (P = 0.0092) for any de novo solid tumour [16].

Proliferation signal inhibitors in the management of cancer

Although there is a considerable amount of data to suggest that use of PSIs is associated with a reduced risk of post-transplant malignancy, there is also some evidence that a switch to a PSI may be beneficial in the management of such malignancies. Elsharkawi et al. [17] report a patient with recurrent bilateral pulmonary metastases after receiving a liver transplant as a result of hepatocellular carcinoma. The patient was switched from CsA-based immunosuppression to a regimen of sirolimus and mycophenolate mofetil, and, 4 months after conversion, no pulmonary metastases were detectable by computed tomography. This finding was confirmed by integrated positron emission tomography and computed tomography, and the patient remained free of metastases 18 months after conversion. Conversion from CNIs to sirolimus has also been shown to lead to resolution of Kaposi’s sarcoma [18,19], with complete regression of lesions observed 6 months after conversion [18]. In agreement with these findings a recently reported case study has shown that withdrawal of sirolimus may be associated with abrupt onset of Kaposi’s sarcoma [20].

In light of these promising results with PSIs in the management of post-transplant malignancy, a clinical oncology programme has been initiated in which everolimus is being evaluated for the treatment of advanced solid tumours. Results have recently been presented from a Phase I study exploring the safety, pharmacokinetics and molecular pharmacodynamics of everolimus (20, 50 and 70 mg/week, or 5 and 10 mg/day) in such patients [21]. The study included 33 patients, with grade 3 dose-limiting toxicity occurring in five (one patient at 10 mg/day and
four patients at 70 mg/week). Partial response was observed in one patient with colon cancer, and disease stabilization (≥4 months) was recorded in one patient with renal cell carcinoma and one patient with breast cancer. Pharmaco-dynamic analysis suggested a dose-dependent effect on molecular targets, including Akt, with the maximum effect at 10 mg/day or ≥50 mg/week. Based on these results, the authors suggest that an everolimus dose of 10 mg/day would be appropriate for further clinical evaluation as a treatment for solid tumours. Furthermore, PSIs are currently being examined in combination therapy with chemotherapy agents. Sirolimus in combination with epithelial growth factor receptor has shown promise in the treatment of gliomas, with a 50% partial response rate and 25% survival-free progression at 6 months [22]. Both everolimus and temsirolimus are being examined in combination with letrozole for the treatment of breast cancer, with pre-clinical and early clinical data suggesting a synergistic effect between PSI and chemotherapy treatment [23,24].

Summary

The PSI class of immunosuppressive drugs clearly has anti-cancer properties as a result of its broad effect on intracellular signalling pathways such as the PI3K-AKT-mTOR pathway, leading to slowing or inhibition of cell-cycle progression, increased sensitivity to apoptosis and reduced angiogenesis. Pre-clinical studies have demonstrated that administration of PSIs can prevent neoplastic cell growth in vitro and in vivo, and clinical trials have shown a reduced incidence of cancer in patients receiving PSIs (with or without a CNI) compared with those receiving a CNI only. Data also suggest that PSIs may have a role in the management, and potentially in the prevention, of cancer, both in transplant recipients and in patients with advanced solid tumours. Further research is needed to establish the precise role of these agents, but there are sufficient data to justify their use in the management, and perhaps also prevention, of a variety of tumours in transplant recipients. Clinical guidance on these issues is essential, to maximize the benefit of PSIs [25].

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