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Potential role of proliferation signal inhibitors on atherosclerosis in renal transplant patients

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Abstract

Over the last decade, there has been a decrease in acute graft rejection rates following renal transplantation; however, this has not corresponded with an improvement in long-term outcomes of transplantation. One of the major causes of long-term morbidity and mortality in renal transplant recipients is cardiovascular disease. Immunosuppressive regimens, especially those including steroids and calcineurin inhibitors, have a negative role in the induction of cardiovascular risk factors. The proliferation signal inhibitors (PSIs)/mammalian target of rapamycin (mTOR) inhibitors sirolimus and everolimus have shown considerable promise in reducing acute rejection in renal transplant recipients. Although PSIs are associated with an increase in hyperlipidaemia (hypercholesterolaemia and hypertriglyceridaemia), which is a major risk factor for atherosclerosis and associated cardiovascular disease, recent studies with sirolimus have demonstrated protection from atheroma progression in hyperlipidaemic apolipoprotein E-deficient mice. Here, we summarize the results of pre-clinical and clinical studies with sirolimus and everolimus, with particular emphasis on the beneficial and adverse effects that these drugs exert on the cardiovascular system, and the underlying molecular mechanisms.

Keywords: atherosclerosis; cardioprotective; dyslipidaemia; everolimus; mammalian target of rapamycin inhibitors; proliferation signal inhibitors; renal transplant; sirolimus

Introduction

Cardiovascular disease is currently one of the leading causes of death in renal transplant recipients. Renal transplantation is associated with an increased risk of cardiovascular disease, with patients commonly experiencing cardiovascular complications such as diabetes mellitus, hypertension and hyperlipidaemia. Furthermore, renal insufficiency and dialysis treatment is associated with the progression of advanced atherosclerotic disease [1]. Whereas some immunosuppressive regimens, such as those including steroids or calcineurin inhibitors, may exacerbate the risk of cardiovascular disease, others may have a cardioprotective role.

The proliferation signal inhibitors (PSIs)/mTOR inhibitors everolimus (Certican®; Novartis Pharma AG, Basel, Switzerland) and sirolimus (Rapamune®; Wyeth Pharmaceuticals, USA) are becoming increasingly used following renal and heart transplantation as a result of their immunosuppressive and antiproliferative actions. Sirolimus is a macrolide antibiotic produced by *Streptomyces hygroscopicus* [2], which displays potent immunosuppressive, antiproliferative and anti-migratory properties [3]. Upon binding to the cytosolic immunophilin FK506-binding protein, sirolimus inhibits the FRAP {FKBP-12 rapamycin-associated protein [also known as mTOR]} signalling pathway, thus promoting dephosphorylation and inactivation of p70 ribosomal protein S6 kinase and eukaryotic translation initiation factor 4E-binding protein, accumulation of the growth suppressor $p27^{Kip1}$, inhibition of cyclin-dependent kinase activity, reduced retinoblastoma protein phosphorylation and inhibition of minichromosome maintenance protein expression [4]. Everolimus also inhibits the phosphatidylinositol-3-kinase signalling pathway, blocking growth factor-mediated interleukin (IL)-2- and IL-15-driven proliferation of T-cells, B-cells and vascular smooth muscle (Figure 1) [5].

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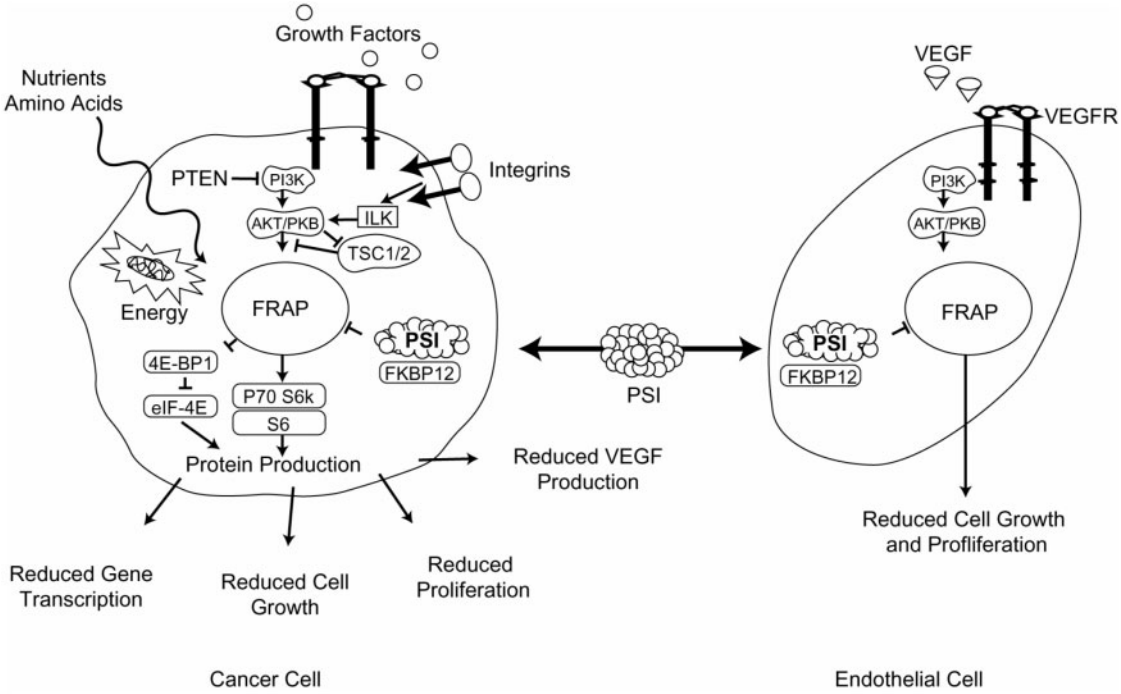

Fig. 1. Mechanism of action of proliferation signal inhibitors. FKBP, FK506-binding protein; FRAP, FKBP-12 rapamycin-associated protein [mTOR]; ILK, integrin-linked kinase; p70 S6k, p70 ribosomal protein S6 kinase; PI3K, phosphatidylinositol 3-phosphate; PKB, protein kinase B; PSI, proliferation signal inhibitor; PTEN, phosphatase and tensin homologue deleted from chromosome 10; TSC, tuberous sclerosis complex; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor.

Although sirolimus and everolimus have similar chemical structures, there are key differences in their pharmacokinetic and pharmacodynamic profiles, and in their tolerability. Everolimus has a shorter half-life than sirolimus (28 h *vs* 62 h, respectively), and the time to reach steady-state is shorter for everolimus (4 days *vs* 6 days, respectively) [6].

Clinical trials

Clinical trials have demonstrated the efficacy of sirolimus and everolimus therapy in reducing acute graft rejection in kidney [7,8], heart [9,10], liver [11,12] and lung [13,14] transplant recipients. Sirolimus and everolimus also attenuate neointimal thickening and transplant atherosclerosis in animal models of angioplasty, and vessel and cardiac allografts [3,15]. Moreover, clinical trials have demonstrated a significant reduction in binary restenosis, late lumen loss and repeat revascularization rates in selected patient groups receiving sirolimus-eluting stents compared with standard coronary stents [3,16]. Preliminary data from the First Use To Underscore REduction in restenosis with everolimus I (FUTURE I) trial suggest that these effects are PSI class specific, with lower rates of in-stent late lumen loss in everolimus-eluting stents compared with metallic stents [17]. Up-regulation of $p27^{Kip1}$, which can reduce both vascular smooth muscle cell proliferation and migration, might mediate, at least in part, the anti-proliferative and anti-migratory actions of sirolimus, thus contributing to reduced neointimal thickening [3,18].

Adverse events in patients receiving sirolimus-eluting stents include increased risk of late stent thrombosis after cessation of anti-platelet therapy [19,20] and exercise-induced paradoxical coronary vasoconstriction of the adjacent vessel segments (although the vasodilatory response to nitroglycerin is unaffected) [21]. These may be due to drug-induced impaired intimal healing and endothelial dysfunction, respectively. In renal transplant recipients, sirolimus induces dose-dependent hyperlipidaemia [e.g. hypertriglyceridaemia, increased low-density lipoprotein (LDL)-cholesterol and increased apolipoprotein (apo) B-100 and apoC-III circulating levels], which can be reversed after discontinuation of treatment and is well-managed with conventional statin therapy [22–24]. A similar increase in serum cholesterol and triglyceride levels has also been reported in renal transplant recipients receiving everolimus therapy [25]. Suggested mechanisms underlying PSI-induced dyslipidaemia in humans are not clear, and may include increased hepatic secretion of triglycerides in conjunction with cholesterol and apoproteins in very-low-density lipoprotein (VLDL)-cholesterol particles and hypertriglyceridaemia. These may occur as a result of augmented adipose tissue lipase activity and/or

decreased lipoprotein lipase activity in response to altered insulin-mediated signalling [26,27], and it has been proposed that sirolimus indirectly up-regulates expression of the gene *apo CIII*, an important inhibitor of lipoprotein lipase [28]. Furthermore, up-regulation of adipocyte fatty acid-binding protein (aP2) expression in monocytes and macrophages may play a role in the increased accumulation of triglycerides [29].

Recent animal studies

Although the above findings suggest that PSI therapy may promote atherosclerosis, since cholesterol commonly forms part of atherosclerotic plaques in coronary artery disease, recent studies using the well-characterized hyperlipidaemic apoE-null mouse model of atherosclerosis [30] have demonstrated diminished atheroma size following sirolimus administration, either subcutaneously [4,31] or orally [32,33]. Notably, sirolimus did not aggravate hypercholesterolaemia in fat-fed apoE-null mice [4,31–33]. Castro *et al*. found no significant effect of sirolimus (1–4 mg/kg, s.c., q.i.d.) on circulating LDL-cholesterol, high-density lipoprotein (HDL)-cholesterol, VLDL-cholesterol and intermediate-density lipoprotein-cholesterol [4]. By contrast, in another study, Elloso *et al*. q.i.d. reported increased levels of LDL-cholesterol and HDL-cholesterol in the sirolimus-treated group (1–8 mg/kg, s.c., q.i.d.) [31]. Likewise, the effect of sirolimus on circulating triglyceride levels in fat-fed apoE-null mice is controversial, with these levels being unchanged upon oral administration of sirolimus but increased upon intraperitoneal sirolimus administration (3 mg/kg/day) [34,35]. Regardless of these discrepancies, which might be related to differences in high-fat diet composition or doses and route of drug administration, it can be concluded that sirolimus protects from atheroma development in mouse models, even in the presence of severe dyslipidaemia. This may be a class effect of PSIs, and further studies are required to elucidate any beneficial effects of everolimus on the development of atherosclerosis.

Mechanisms of action

Several mechanisms may contribute to sirolimus-dependent atheroprotection regulating both cholesterol homeostasis and inflammatory responses. Firstly, there may be reduced accumulation of cholesterol within the artery wall [36], possibly owing to reduced expression of lipoprotein receptors (LDL receptors, VLDL receptors and CD36) and increased expression of genes involved in cholesterol efflux, including the genes encoding peroxisome proliferator-activated receptor-α, liver X receptor-α and ATP-binding cassette A1 [37]. This results in increased cholesterol efflux from cells, leading to lower intracellular cholesterol levels. Furthermore, there may also be alterations in endothelial nitric oxide synthase expression, changes in the ratio of matrix metalloproteinases (MMP) to tissue inhibitors of metalloproteinases and MMP-2 and MMP-9 activation [34,35], reduced expression of the positive cell cycle regulator cyclin-dependent kinase-2 and proliferating cell nuclear antigen [4] and pro-inflammatory cytokines [31], and diminished expression of arterial monocyte chemotactic protein-1 [4], which is associated with the attenuation of monocyte chemotaxis and reduced neointimal macrophage counts in sirolimus-fed mice [32]. A study with everolimus has demonstrated that everolimus can cause disruption of cellular lipid homeostasis in mouse peritoneal macrophages at concentrations similar to therapeutic range (0.01 μM), resulting in increased cholesterol esterification and efflux and reduced triglyceride biosynthesis [38].

Conclusions

Whether the beneficial actions of PSIs/mTORs observed in severely dyslipidaemic mice also operate in renal transplant recipients, and whether they might counteract PSI-induced hyperlipidaemia is, as yet, unknown. Certainly, prospective long-term trials are needed to clarify the impact of sirolimus and everolimus on lipid and lipoprotein patterns and on morbidity and mortality attributable to cardiovascular disease. In short, the intrinsic effect on atherosclerosis prevention and enhanced control of renal function could explain the reduction of cardiovascular morbidity and mortality in PSI-treated patients.

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