Editorial

Bench to Bedside

The Development of Rapamycin and Its Application to Stent Restenosis

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In response to physiological stimuli (eg, wound healing), normally quiescent smooth muscle cells (SMCs) within the vessel wall can be activated to migrate and proliferate to produce new blood vessels. In addition to this physiological response, pathological migration and proliferation within the vessel wall can occur in disease states. Examples of such disease states include tumor growth and metastasis, diabetic retinopathy, arthritis, accelerated arteriopathy after cardiac transplantation, and neointimal proliferation after balloon angioplasty (PTCA) and stent placement. An important limitation of PTCA is restenosis, which is due in large part to luminal narrowing; restenosis occurs in 20% to 40% of patients within the first few months after a successful intervention.12 The percentage of patients that develop early restenosis after PTCA can be reduced by stent implantation. However, stents actually increase the amount of late luminal narrowing due to intimal hyperplasia,2 and the overall rate of stent restenosis remains unacceptably high (~30%).

Numerous pharmacological agents, including antiplatelet agents, anticoagulants, ACE inhibitors, and cytotoxic agents, have failed to adequately reduce restenosis after PTCA and stenting. Novel therapeutic approaches based on understanding the molecular mechanisms that cause intimal hyperplasia are needed to reduce the high incidence of stent restenosis. Arterial injury is associated with SMC activation and re-entry into the cell cycle. Multiple approaches to inhibiting SMC proliferation have been and are being evaluated. Gene delivery systems aimed at blocking SMC proliferation have been tested4,5; however, the low efficiency and/or potential hazards of this approach may limit its usefulness. Radiation therapy has been evaluated in numerous studies and has shown considerable promise as a nonpharmacological, antiproliferative approach for the reduction of restenosis. However, significant side effects, including late stent thrombosis, may also limit its usefulness.6–8 The unusually late stent thrombosis seen in the patients treated with radiation therapy suggests that radiation may impair the ability of the vessel wall to endothelialize the stent struts.9

Recently, much attention has focused on the potential use of rapamycin (Sirolimus) to prevent stent restenosis. Rapamycin evolved from a failed antibiotic with no apparent therapeutic utility to its current status as a promising cardiovascular drug on the basis of a series of laboratory studies revealing that it is a potent inhibitor of both SMC proliferation and migration.10,11 Rapamycin, a macrolide antibiotic, is a natural fermentation product produced by Streptomyces hygroscopicus, which was originally identified in a soil sample from Rapa Nui (Easter Island) by the Canadian Medical Research Expedition (December 1964 through February 1965).12,13 Rapamycin was originally noted to have antifungal properties and, subsequently, its potent immunosuppressant properties (which made it unsuitable for use as an antibiotic) were appreciated.13 Largely because rapamycin was viewed as an immunosuppressant drug, its potential application for other therapeutic targets, including accelerated arteriopathy after cardiac transplantation and stent restenosis, remained underappreciated for almost a decade. The emergence of other promising anti-restenosis therapies further suppressed interest in rapamycin, despite accumulating in vitro evidence indicating that it has properties that are potentially ideally suited to attack important cardiovascular diseases.10,11,14–20

Rapamycin’s cellular actions are mediated by binding to its intracellular receptor, the FK506 binding protein (FKBP12), a member of the immunophilin family of proteins.21 The related immunosuppressant drug FK506 (tacrolimus) also binds to FKBP12, but this complex inhibits the phosphatase calcineurin. FK506-FKBP12 has no antiproliferative or anti-migratory activity in vascular SMCs.10,11 Rapamycin-FKBP12 has no activity against calcineurin; rather, it inhibits a kinase called the target of rapamycin (TOR),22 which is a component in a pathway that regulates cell cycle progression (Figure 1). The finding that rapamycin inhibits multiple fundamental regulators of cell cycle progression in vascular SMCs suggested that it might have utility in the prevention of diseases linked to vascular SMC proliferation.10

We and others demonstrated that rapamycin inhibits the proliferation of rodent and human vascular SMCs in vitro and porcine vascular SMCs in vivo by blocking cell cycle progression at the G1/S transition.10,16,19 These studies in vascular SMCs were based on earlier observations showing that the immunosuppressant activity of rapamycin was linked to its ability to inhibit cell cycle progression in T lymphocytes.23–25 In addition, we showed that rapamycin had the unexpected property of inhibiting vascular SMC migration: treating rat and human vascular SMCs with rapamycin (2 nmol/L) for 48 hours inhibited platelet-derived growth factor–induced migration in a modified Boyden chamber.11

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These antiproliferative and antimigratory properties were specific to rapamycin; FK506 failed to inhibit either vascular SMC proliferation or migration. In fact, FK506 antagonizes rapamycin’s antiproliferative and antimigratory properties\(^\text{10,11}\) because both agents bind to the same cytosolic receptor, FKBP12.\(^\text{26}\) The findings that rapamycin possessed both antiproliferative and antimigratory properties led to the proposal that rapamycin should be tested in the treatment of disorders, such as accelerated arteriopathy, that occur in transplanted hearts and restenosis after PTCA and placement of coronary stents.\(^\text{10,11}\)

Further investigation of the mechanisms by which rapamycin inhibits vascular SMC proliferation and migration provided additional support for its potential role as a cardiovascular therapeutic agent. Rapamycin-induced inhibition of vascular SMC proliferation is associated with a marked reduction in cyclin-dependent kinase (CDK) activity and a reduction in retinoblastoma protein phosphorylation.\(^\text{10}\) These actions of rapamycin in vascular SMCs are similar to its actions in other cell lines.\(^\text{25,27}\)

The development of a rapamycin-resistant muscle cell line allowed us to elucidate the mechanism by which rapamycin inhibits vascular SMC proliferation. Using rapamycin-resistant muscle cell lines, we showed that the CDK inhibitor p27kip1 mediates rapamycin’s antiproliferative and antimigratory properties in muscle.\(^\text{17,20}\) Cell cycle arrest in some cell types is linked to elevation of p27kip1.\(^\text{17,20}\) p27kip1 increases in response to mitogen deprivation, cell-cell contact, or transforming growth factor-\(\beta\), whereas p27kip1 levels decrease in response to mitogens.\(^\text{28}\) Rapamycin inhibits mitogen-induced downregulation of p27kip1.\(^\text{23}\) We showed that the defect in rapamycin-resistant muscle cells that allowed them to escape the antiproliferative effects of rapamycin was constitutively low levels of p27kip1\(^\text{27}\) and the inability to increase p27kip1 in response to rapamycin.\(^\text{17}\) These findings were confirmed using cells from p27kip1-null mice\(^\text{29}\) that exhibited partial rapamycin resistance.\(^\text{17}\) Rapamycin also inhibits p70S6k phosphorylation and activity,\(^\text{30}\) as well as phosphorylation of the eukaryotic translation initiation factor 4E-binding protein in vascular SMCs.\(^\text{31}\)

These findings suggested that part of the antiproliferative effect of rapamycin is linked to inhibition of protein translation. However, although rapamycin blocks phosphorylation of p70S6k in p27kip1-null cells\(^\text{17}\) and inhibits 4E-binding protein phosphorylation in the rapamycin-resistant muscle cells,\(^\text{32}\) the cells continue to proliferate. These findings further support the conclusion that it is the inability to elevate p27kip1 levels that attenuates the antiproliferative properties of rapamycin and that regulation of p27kip1 is a critical mechanism by which rapamycin inhibits vascular SMC growth. Moreover, we recently reported that the lack of p27kip1 also reduces rapamycin-mediated inhibition of vascular SMC migration, thus implying that p27kip1 has an important role in the signaling pathway(s) regulating SMC migration.\(^\text{29}\) In agreement with our studies, others have shown that overexpression of p27kip1 in SMCs inactivated cdk2 and cdk4 activity and that adenoviral gene transfer of p27kip1 after femoral artery balloon angioplasty significantly inhibited intimal cell proliferation.\(^\text{33}\)

In September 1999, rapamycin was approved by the US Food and Drug Administration as an agent to prevent acute rejection in renal transplant patients. It is important to emphasize that the immunosuppressant activity of rapamycin is mediated through a mechanism distinct from that of FK506 and cyclosporine A (both of which inhibit calcineurin). The therapeutic benefits of both cyclosporine A and FK506 are limited, to some extent, by acute and chronic nephrotoxicity, which is believed to be linked to calcineurin inhibition. In contrast, because of its distinct mechanism of action, rapamycin use is not associated with nephrotoxicity.\(^\text{34}\) Regular administration of rapamycin (particularly in transplantation patients) produces other side effects in humans, including headaches, polyarthralgia, mild stomatitis, epistaxis, diarrhea, skin complaints, myelosuppression, hyperlipidemia, and overimmunosuppression.\(^\text{34,35}\)

Indeed, the implantation of rapamycin-coated stents (BX Velocity) in de novo lesions was recently shown to be safe and effective in inhibiting neointimal formation\(^\text{36}\) in 30 patients with stable and unstable angina. Half of the patients received the fast-release formulation designed to deliver the drug (140 \(\mu\)g/cm\(^2\)) within 15 days, and half received the slow-release formulation (\(\geq\)28 day drug release). Patients...
also received clopidogrel (75 mg/d) for 60 days after stent implantation. No patient approached >50% vessel narrowing by intravascular ultrasound or quantitative coronary angiography, and only 3 patients had >15% intimal hyperplasia by intravascular ultrasound at 4 months of follow-up.36 Although the number of patients studied was small, no edge restenosis or stent thrombosis was observed, both of which have been reported in studies of patients undergoing radiation therapy during coronary intervention.8,37 No adverse events were reported after 8 months of follow-up.36 Similar results were observed for both release formulations, although preliminary results suggest that the excessive proliferation and migration of vascular SMCs after coronary intervention can be prevented with ≤15 days of intracoronary rapamycin exposure.36 At longer follow-up (6 months in 13 patients treated in Rotterdam and 12 months in 27 patients treated in Sao Paulo), the reduction in ultrasound-measured intimal hyperplasia in patients treated with rapamycin-coated stents persisted.38,39 These data suggest that rapamycin may provide protection against intimal hyperplasia after stent implantation in coronary arteries and, potentially, in peripheral arteries, without the complications seen with other modalities that reduce the incidence of restenosis.

The use of rapamycin to prevent chronic graft vascular disease (CGVD) or accelerated arteriopathy, especially after cardiac transplantation, may be an additional target for the drug’s antiproliferative and antiangiogenic properties. CGVD is marked by progressive development of coronary artery narrowing (concentric neointimal hyperplasia), possibly due to an ill-defined immunological response. Despite immunosuppression with cyclosporine A or FK506, CGVD persists, as manifested by progressive narrowing of the coronary arteries in the transplanted heart in a diffuse pattern that occurs in up to 75% of patients within the first year and affects virtually 100% of patients by year 4 to 5 after cardiac transplantation.40 Rapamycin’s potent immunosuppressive properties and the findings that rapamycin, but not FK506, inhibited vascular SMC proliferation and migration in vitro suggest that rapamycin would be the agent of choice for immunosuppression in cardiac transplantation patients.10,41 Moreover, rapamycin reversed the development of CGVD in a rodent heart allograft model.42 Indeed, rapamycin and the closely related compound 40-O-(2-hydroxyethyl) RAD (everolimus) are currently being investigated in clinical trials to determine whether they can inhibit intimal hyperplasia in cardiac transplant recipients.

References

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