Periadventitial Rapamycin-Eluting Microbeads Promote Vein Graft Disease in Long-Term Pig Vein-Into-Artery Interposition Grafts

Thirumaran Rajathurai, BMedSci, BM, BS; S. Imran Rizvi, MD, MRCS; Hua Lin, MSc; Gianni D. Angelini, MD, FRCS; Andrew C. Newby, PhD; Gavin J. Murphy, MD, FRCS

Background—Neointima formation and atherosclerosis compromise long-term graft patency in aortocoronary and peripheral vein bypass grafts. We investigated the short- and long-term effects of periadventitial application of a sustained-release formulation of rapamycin on experimental pig vein grafts with similar dimensions and kinetics to human saphenous vein bypass grafts.

Methods and Results—Periadventitial application of rapamycin-eluting polyvinyl alcohol microspheres (60 pg cm⁻²) to porcine saphenous vein-to-carotid artery interposition grafts inhibited vein graft positive and vascular smooth muscle cell proliferation in 1-week grafts. It also decreased neointima formation and wall thickening in 4-week vein grafts compared with controls. The inhibition of vein graft thickening was not sustained; however, a catch-up phenomenon was observed, and there was no therapeutic benefit evident in 12-week grafts. Increasing the dose of rapamycin to 120 pg cm⁻² was associated with significant local toxicity manifest by high rates of graft rupture (25%), inhibition of adventitial neoangiogenesis, and a paradoxical acceleration of vein graft disease as evidenced by increased vascular smooth muscle cell proliferation.

Conclusions—Local toxicity and poor long-term efficacy limits the clinical applicability of locally applied, sustained rapamycin release in vein graft disease. (Circ Cardiovasc Interv. 2010;3:00-00.)

Key Words: drugs ▪ microspheres ▪ muscle ▪ smooth ▪ vein bypass ▪ atherosclerosis

The success of coronary artery bypass grafting is limited by poor long-term graft patency. Despite the superior patency of arterial grafts, saphenous vein remains the most commonly used conduit for coronary artery bypass because of its predictable handling qualities and ready availability. More than 40% of vein grafts are thrombosed at 10 years postoperatively, largely as a consequence of vein graft disease that is characterized by neointima formation, atherosclerosis, plaque rupture, and graft thrombosis. Graft failure results in major adverse cardiac events and leads to repeat revascularization procedures. With the exception of aggressive lipid lowering, no therapy has been shown to improve long-term vein graft patency in clinical studies. Graft failure also occurs in peripheral bypass grafts, with primary graft patency rates at 1 year as low as 60%.

Clinical Perspective on p

We and others have shown that inhibition of early neointima formation in experimental vein grafts inhibits subsequent foam cell accumulation and atherogenesis. Strategies that have been shown to inhibit vein graft disease in experimental studies such as placement of external porous Dacron stents or perivascular application of decoy oligonucleotides have failed to translate into clinical benefits because of early graft thrombosis and poor efficacy, respectively. We have recently evaluated the potential for local application of antiproliferative agents to vein grafts at the time of surgery as a means of inhibiting later vein graft disease. Immersion of vein grafts in rapamycin solution immediately before grafting inhibits neointima formation in porcine vein grafts; however, this effect is not sustained. For this study, we hypothesized that perivascular application of a slow-release formulation of rapamycin might produce a more sustained antiproliferative effect and would avoid high drug tissue levels that could cause local toxicity. We therefore measured the short- and long-term effect of periadventitial application of rapamycin-eluting polyvinyl alcohol (PVA) microspheres at 2 doses, with vein graft thickening as a primary end point and evidence of local and systemic toxicity as a secondary measure.

Methods

Animals

A total of 56 large white-Landrace cross pigs weighing 28.2 ± 1.0 kg were used. All procedures had local ethical approval, were performed under UK government license (Animals [Scientific Procedures] Act 1986), and conform to the Guide for the Care and Use of Laboratory Animals.

Clinical Perspective on p

We and others have shown that inhibition of early neointima formation in experimental vein grafts inhibits subsequent foam cell accumulation and atherogenesis. Strategies that have been shown to inhibit vein graft disease in experimental studies such as placement of external porous Dacron stents or perivascular application of decoy oligonucleotides have failed to translate into clinical benefits because of early graft thrombosis and poor efficacy, respectively. We have recently evaluated the potential for local application of antiproliferative agents to vein grafts at the time of surgery as a means of inhibiting later vein graft disease. Immersion of vein grafts in rapamycin solution immediately before grafting inhibits neointima formation in porcine vein grafts; however, this effect is not sustained. For this study, we hypothesized that perivascular application of a slow-release formulation of rapamycin might produce a more sustained antiproliferative effect and would avoid high drug tissue levels that could cause local toxicity. We therefore measured the short- and long-term effect of periadventitial application of rapamycin-eluting polyvinyl alcohol (PVA) microspheres at 2 doses, with vein graft thickening as a primary end point and evidence of local and systemic toxicity as a secondary measure.
Rapamycin-Eluting Microspheres

Microspheres (gifted from Dr Andrew Lewis, Biocompatibles, Farnham, Surrey, England) are modified PVA hydrogel spheres 200 μm in diameter. Drug-eluting microspheres were loaded with rapamycin by a proprietary technique. The formulation chosen to perform the experiments eluted rapamycin for up to 9 days (Figure 1). This period corresponds to maximal medial vascular smooth muscle cell (VSMC) proliferation in the porcine vein graft model. Microspheres containing 19 mg of rapamycin were hydrated with 1 mL of H₂O, suspended in 1 mL of Pluronic-F 127 gel to give the appropriate concentration, and applied to the surface of the vein graft with a syringe after completion of the distal anastomoses. An initial dose was selected corresponding to 60 mg/cm² of the adventitial surface of the vein graft. Similar dose ranges were used in the preclinical evaluation of rapamycin-eluting stents in porcine coronary arteries. In a separate study of 4-week grafts, we also compared non–drug-eluting microspheres hydrated as before and suspended in Pluronic-F 127 gel versus Pluronic-F 127 alone (n=8 pigs) to determine whether a response to the beads may have influenced our results.

Porcine Autologous Saphenous Vein-to-Carotid Artery Interposition Grafts

The method of the saphenous vein-to-carotid artery interposition grafting model has been described previously. Animals were anesthetized with ketamine (Ketaset, 100 mg/mL) and halothane, intubated, and allowed to spontaneously ventilate. The saphenous vein was harvested from the hind leg, the animal was heparinized by intravenous administration of 100 IU/kg of heparin, and a 3-cm length of vein was grafted as an interposition graft to the internal carotid artery using continuous 7/0 Surgipro sutures bilaterally. Rapamycin-eluting or non–drug-eluting microspheres were randomly allocated to either the right or the left vein graft, with 1-mL Pluronic-F 127 gel applied to the contralateral graft as a paired control. Animals were recovered, returned to their pen, and fed a normal chow diet for the duration of the experiment. Grafts were harvested at 1, 4, or 12 weeks. Only patent grafts were used for subsequent analyses.

Histological Methods

Vein grafts were pressure fixed at 100 mm Hg with 4% formalin in PBS, wax embedded, and sectioned into 4-μm transverse sections. Four transverse sections at equally spaced intervals along the graft length were stained with Miller’s elastic van Gieson stain. For each section, the luminal margin and internal and external elastic laminae were identified and traced from digital images, and total vessel area (area within external elastic lamina); neointimal, medial, and total wall areas (intima plus media); and luminal area were calculated using image-analysis software as described previously. The ratio of the lumen radius to the wall thickness, an index of the wall stress was also calculated.

Cell proliferation was measured by proliferating cell nuclear antigen (PCNA) immunocytochemistry (ICC) staining as previously described. The total numbers of cells positive for PCNA were counted in 4 fields at ×40 magnification, which abutted the lumen and included the neointima and inner media. Four sections per graft were assessed. The number of PCNA-positive cells was expressed as a percentage of the total cell number (PCNA index).
Evaluation of endothelial coverage within grafts was achieved using an ICC stain for biotinylated dolichos biflorus agglutinin lectin as previously described. Endothelial coverage was calculated as an average score over 4 sections per graft. The endothelial score was defined by the proportion of luminal coverage with endothelial cells staining positive (1 = 33%; 2 = 33% to 66%; 3 = 66%; 4 = 100%). Previous work has established an inverse correlation between formation of the vasa vasorum and neointimal growth in this model, probably as a result of vessel wall hypoxia. Neangiogenesis within the graft wall was determined by calculating the mean number of dolichos biflorus agglutinin lectin–stained microvessels as counted in 4 fields at ×10 magnification in 4 sections per graft. Apoptosis was assessed by ICC staining using rabbit antimonous-cleaved caspase-3 antibody, and inflammatory cell infiltration was determined by ICC for MAC387 antibody, with staining and quantification as per the PCNA protocol.

**Measurement of Rapamycin Levels**

Rapamycin levels were measured by reverse-phase high-performance liquid chromatography as previously described. Briefly, whole-blood samples (1 mL) or homogenized vein graft tissue were treated with precipitation reagent (methyl tert-butyl ether:1-chloroethane:methanol, 45:45:10) plus internal standard solution (32–0-desmethoxysirolimus) followed by C18 solid-phase extraction. UV detection of the absorbance peaks was performed at 278 nm, and values were expressed as micrograms per gram of dry tissue.

**Power Calculations and Statistical Analysis**

Neointimal area at 4 weeks was our primary end point. We calculated that a study with 6 animals per group would have a 90% power to detect a 50% reduction in neointimal area relative to contralateral paired controls (equivalent to a difference of 2.1 mm², assuming a within-group SD of 1.4 mm²). To account for animal losses in this recovery model, up to 8 animals were randomly allocated to each group. We detected a crossover effect of rapamycin in contralateral controls at the high dose and were unable to perform paired comparisons; therefore, we compared each intervention group to low-dose controls where no crossover was detected. With 8 animals per group, the calculated power was reduced to 80%. We did not power the study for other measures or adjust for repeated measures, and these comparisons should be considered as exploratory. Categorical data were compared using χ² tests with Fisher exact test where expected values were <5. Continuous data were compared using unpaired Student t tests. Effect sizes are expressed throughout as the percentage difference and the mean difference (95% CI). Values were considered significant if P was <0.05.

**Results**

**Rapamycin Pharmacokinetics**

Measurement of rapamycin levels in vein grafts demonstrated rapamycin in low-dose–treated vein grafts at 7 days but not at 28 days after grafting (Figure 1). Conversely, rapamycin was detected in high-dose–treated vein grafts at both 7 and 28 days. Rapamycin was not detected in contralateral low-dose control grafts at any time point but was detected in contralateral high-dose control grafts at 7 days but not at 28 days (Figure 1). This finding indicates a crossover effect in the high-dose pigs that was not evident in the low-dose pigs. Rapamycin was not detected with high-performance liquid chromatography in whole-blood samples of any study animal at 1 week after grafting, suggesting that this crossover may have arisen from lymphatic or local diffusion. Based on these results, we rejected high-dose control vein grafts as acceptable controls and elected to compare all interventions with low-dose control vein grafts.

**Effects of Low-Dose Rapamycin Microspheres on Vein Graft Morphology**

Our initial experiments evaluated rapamycin-eluting microspheres at a dose of 60 μg·cm⁻². In 1-week vein grafts, this dose significantly decreased total vessel area by 51% (mean difference, 15.99 mm²; 95% CI, 4.59 to 27.41; P=0.011) and lumen area by 54% (mean difference, 16.0 mm²; 95% CI, 4.04 to 27.97; P=0.014) relative to contralateral untreated controls (Figure 2A and 2B). Neither intimal area nor total wall area was affected at this early time point (Figure 2C and 2D), and there was no significant difference in the ratio of the lumen radius to the wall thickness (online-only Data Supplemental Figure IA). We concluded that low-dose rapamycin inhibited early positive remodeling of the vein graft wall. Rapamycin microspheres significantly reduced medial VSMC proliferation measured by PCNA staining in...
1-week vein grafts (mean difference, 19.66%; 95% CI, 4.60 to 34.7; \( P = 0.016 \)) relative to untreated controls (Figure 3).

In 4-week grafts, rapamycin 60 \( \mu \text{g} \cdot \text{cm}^{-2} \) had no effect on vessel or lumen area (Figure 2A and 2B), which implies that the early effect on remodeling was overcome. In this pig model, the main period of neointima formation is between 1 week and 4 weeks after grafting, a pattern that was reproduced here in low-dose control grafts (Figure 2D). The early inhibition of VSMC proliferation in rapamycin-treated grafts was translated into a significant 63% reduction in neointima formation (mean difference, 2.86 mm\(^2\); 95% CI, 0.13 to 5.59; \( P = 0.041 \)) in 4-week grafts (Figure 2D). Reductions in medial area (mean difference, 3.27 mm\(^2\); 95% CI, -0.39 to 6.96; \( P = 0.077 \)) and overall wall area (mean difference, 6.14 mm\(^2\); 95% CI, -0.19 to 12.48; \( P = 0.057 \)) failed to reach conventional statistical significance (Figures 2C and 4A and 4B). VSMC proliferation (PCNA index) declined as expected between the 1-week and 4-week vein grafts (Figure 3A). Furthermore, rapamycin 60 \( \mu \text{g} \cdot \text{cm}^{-2} \) no longer had any effect on VSMC proliferation in 4-week grafts (Figure 3A), consistent with its absence in vein graft tissue as measured by high-performance liquid chromatography, and a catch-up was observed in vessel areas in 12-week grafts. At this time point, there were no differences in vessel or luminal area and neointimal, medial, or total wall area in grafts treated with rapamycin 60 \( \mu \text{g} \cdot \text{cm}^{-2} \) compared with controls (Figures 2A through 2D, 4C, and 4D). Based on these findings, we evaluated a higher rapamycin dose (120 \( \mu \text{g} \cdot \text{cm}^{-2} \)) in an attempt to maintain the early antiproliferative effect seen at the 60 \( \mu \text{g} \cdot \text{cm}^{-2} \) dose.

Effects of High-Dose Rapamycin-Eluting Microspheres on Vein Graft Morphology

Consistent with our observation of a crossover effect of rapamycin from high-dose treatment microspheres to the contralateral control grafts, there was no difference between treatment with rapamycin 120 \( \mu \text{g} \cdot \text{cm}^{-2} \) compared with controls (Figures 2A through 2D, 4C, and 4D). Based on these findings, we evaluated a higher rapamycin dose (120 \( \mu \text{g} \cdot \text{cm}^{-2} \)) in an attempt to maintain the early antiproliferative effect seen at the 60 \( \mu \text{g} \cdot \text{cm}^{-2} \) dose.

---

**Figure 3.** A, Graph demonstrating levels of VSMC proliferation in vein grafts treated with low-dose (60 \( \mu \text{g} \cdot \text{cm}^{-2} \)) or high-dose (120 \( \mu \text{g} \cdot \text{cm}^{-2} \)) rapamycin-eluting microspheres at 1 and 4 weeks. \( * P < 0.05 \). \( ** P < 0.001 \). Representative photomicrographs of 4-week vein grafts sections stained for PCNA are as follows: low-dose treatment (B), low-dose control (C), high-dose treatment (D), and high-dose control (E). Bar represents 200 \( \mu \text{m} \).
mean difference, 53.36%; 95% CI, 42.79 to 63.92; \( P < 0.0001 \)) relative to low-dose controls (Figure 3). Rapamycin 120 \( \mu g \cdot cm^{-2} \) resulted in significant thinning of the vessel wall in 1-week grafts, with a 50% reduction in neointimal area (mean difference, 0.18 mm\(^2\); 95% CI, 0.04 to 3.29; \( P = 0.019 \)) and a 34% reduction in total wall area (mean difference, 1.08 mm\(^2\); 95% CI, 0.02 to 2.42; \( P = 0.047 \)) (Figure 2C and 2D) compared with low-dose controls. High-dose rapamycin reduced the ratio of the lumen radius to the wall thickness relative to low-dose controls at 1 week (supplemental Figure IA); however, these differences were not statistically significant (mean difference, 82.9; 95% CI, 12.1 to 177.9; \( P = 0.077 \)). High-dose control grafts, where we detected significant rapamycin levels, also demonstrated significant reductions in VSMC proliferation (Figure 3A).

These differences were not sustained, and there was no difference in neointimal area and total wall area in 4-week high-dose rapamycin-treated grafts compared with low-dose controls (Figures 2C, 2D, 4E, and 4F). In complete contrast to the levels of proliferation at 1 week, we also observed that by 4 weeks, VSMC proliferation in high-dose treatment vein grafts was approximately twice that in low-dose control vein grafts (mean difference, 18.48%; 95% CI, 8.56 to 28.41; \( P = 0.001 \)) and despite the persistent presence of rapamycin in the high-dose treatment vein grafts (Figure 3). There was a reduction in vein graft adventitial neoangiogenesis at the higher dose (vessel count mean difference, 10.8; 95% CI, 22.76 to 1.04) compared with low-dose control grafts (Figures 5A and 6), although this was of not statistically significant (\( P = 0.071 \)). High-dose control grafts also demonstrated significant increases in VSMC proliferation relative to low-dose controls at 4 weeks (Figure 3) and a reduction in angiogenesis (vessel count mean difference, −13.5; 95% CI, −27.19 to 0.98; \( P = 0.051 \)) (Figure 5).
Adverse Events and Toxicity

There was no evidence of systemic toxicity and no difference between baseline weights and in the rate of weight gain between high- and low-dose treatments or in markers of renal and liver injury (supplemental Figure II). There were 8 deaths from graft rupture in the study (2 of 24 in the low-dose group and 6 of 24 in the high-dose group; \( P = 0.124 \)). Graft rupture typically occurred at 5 to 7 days after grafting. At postmortem, it was difficult to determine with certainty the site of rupture in all cases due to edema and hemorrhage. In 4 cases, the rupture site was identified as being immediately adjacent to the venous side of the vascular anastomoses in rapamycin-treated grafts. Ruptured grafts degraded after death and during processing to the point where histological analysis was impossible. These mortality rates were much greater than we have observed previously with this model, typically 3% (\( P < 0.001 \)). An additional 2 animals, (1 low dose, 1 high dose) with patent grafts were culled prematurely for distress and excluded from the analysis. Graft thrombosis, another measure of adverse outcome, occurred in 0 of the 38 control grafts, 3 of the 21 low-dose grafts, and 2 of the 17 high-dose grafts. Increased rates of graft thrombosis in rapamycin-treated vein grafts approached statistical significance (\( P = 0.054 \)). A strong trend, therefore, was seen toward an adverse outcome, including acute graft rupture and graft thrombosis, with the rapamycin-eluting microspheres.

Dolichos biflorus agglutinin lectin staining was used to measure luminal endothelial coverage in grafts and indicated a high level of endothelialization in 1- and 4-week vein grafts with no significant effect of low- or high-dose periadventitial rapamycin-eluting microspheres (supplemental Figure IB). There were no significant differences between the groups in apoptosis rates as determined by ICC staining for cleaved caspase-3 antibody (supplemental Figure IC). There was no difference in intragraft MAC387 antibody staining (macrophages and neutrophils) among any of the groups at 1 week (supplemental Figure ID).

No evidence of local toxicity was attributable to the microspheres. To determine whether a local effect of the microbeads may have influenced our results, we compared non–drug-eluting microspheres in Pluronic gel versus Pluronic alone in 4-week
grafts in a separate study (n = 6). We demonstrated no effect on any measure of vein graft morphometry (neointima, medial, or wall thickness) or on graft remodeling (Figure 6). There was, however, evidence of inflammatory cells in the proximity of the non–drug-eluting beads on hematoxylin/eosin staining that was not present in the vicinity of drug-eluting microspheres at any time point (Figure 6).

Discussion

Main Findings

In our pig model, periadventitial application of rapamycin-eluting microspheres at a dose of 60 μg · cm⁻² inhibited positive vein graft remodeling and VSMC proliferation in 1-week grafts. It also decreased neointima formation and wall thickening in 4-week vein grafts compared with controls. The inhibition of vein graft thickening at this dose was not sustained, and there was no therapeutic benefit evident in 12-week grafts. At a higher dose (120 μg · cm⁻²), rapamycin remained in the tissues for up to 28 days, which was associated with significant local toxicity. High-dose–treated vein grafts demonstrated significant wall thinning at 7 days postgrafting, and there was unacceptably high rates (25%) of graft rupture. In surviving grafts, significant rebound VSMC proliferation in the vessel wall at 28 days and accelerated vein graft disease were found, which may have occurred either as a response to local toxicity, increased wall tension in the thinner-walled vein grafts, or later wall ischemia due to rapamycin-mediated inhibition of adventitial neovascularization.

Study Strengths and Limitations

Our experience with the porcine model suggests that contralateral paired controls are the most accurate in terms of evaluating a treatment effect because they control for differences in animal size and growth. In this study, we demonstrated that a local crossover effect occurred at the higher dose of rapamycin. The mechanism for this crossover effect may have occurred through local diffusion, although each graft is accessed by a separate lateral neck incision, and dissection is kept to a minimum to prevent communication between the 2 graft sites or through lymphatic transmission. A systemic effect is not excluded by our findings, but we were unable to detect rapamycin in blood samples at 1 week, and there was no evidence of systemic toxicity. To overcome this crossover effect, we elected to compare all treatments to low-dose controls where no rapamycin was detected at 7 days. This finding is supported by the similarity between vein graft dimensions in low-dose controls in the rapamycin-eluting microsphere study with those measured in the non–drug-eluting bead study. Nonetheless, there is no certainty that rapamycin was not present in the low-dose control grafts before this time point. The possibility of crossover reduces the power of our observations and represents a limitation of the study. In addition, because we had planned this study to be a preclinical evaluation, it was designed to compare the intervention (rapamycin plus microspheres) to current treatment, which is no treatment. We therefore are unable to determine with certainty whether our effects were attributable to rapamycin alone or whether the delivery system, the PVA microspheres, contributed to our findings. To address this limitation, we
performed a second study comparing non–drug-eluting microbeads plus Pluronic gel to Pluronic gel alone. This second study confirmed that the microbeads had no effect at 4 weeks on vein graft morphometry. Further, the high-dose control grafts, which had detectable rapamycin at 7 days but were not exposed to the PVA microspheres, demonstrated similar morphometry, VSMC proliferation, and neoangiogenesis as the high-dose treatment grafts (rapamycin plus microbeads). This finding suggests that our observations were attributable solely to rapamycin toxicity but was not addressed specifically in the original design and represents a second limitation of the study.

To our knowledge, this study is the first to investigate the long-term effects of local periadventitial application of sustained-release rapamycin in an experimental model with significant homology to vein graft disease in humans. Porcine vein grafts have comparable diameter and wall thickness and are exposed to similar hemodynamic stresses as human vein grafts and develop neointimal thickening over a comparable time frame of 3 to 6 months.20,21 Our findings are at odds with several previous studies that have reported benefits with local rapamycin treatment for the prevention of vein graft disease.20,22 Schachner et al20 demonstrated inhibition of neointima formation after local rapamycin application in the mouse vein graft model. Vein grafts in rodents are much smaller than human or porcine vein grafts, with thinner walls and much higher rates of medial cell necrosis.7,20 The neointima also develops over a much shorter time frame of up to 4 weeks.22,23 Mouse and rabbit models, therefore, are poorly suited to determine diffusion of adventitiously applied drugs or long-term efficacy. This limitation was highlighted by the Prevention of Recurrent Venous Thromboembolism III and IV studies, where E2F antisense oligonucleotides found to be effective at reducing neointima formation in rabbit vein grafts at 4 weeks were ineffective in coronary artery vein bypass grafts and peripheral vein bypass grafts in randomized clinical trials.5,9 Kawatsu et al21 reported a reduction of neointima: total wall area ratios in canine vein grafts at 4 weeks after periadventitial application of rapamycin dissolved in Pluronic gel. Rapamycin exposure using this technique is relatively short. Pluronic gel is rapidly absorbed (24 to 28 h) in vivo, and lipid soluble drugs have a short half-life in tissues, typically less than 24 h.24 Wall thickening in canine vein grafts occurs over a similar period to that in porcine grafts.25 We have shown previously that short-term rapamycin exposure in porcine grafts is effective at 4 weeks but not thereafter.10 Our current findings underline the importance of determining long-term efficacy and toxicity in appropriate preclinical models when evaluating novel therapeutic strategies for the prevention of vein graft disease.

It is interesting to compare our findings to a recent study that examined the long-term efficacy of rapamycin-eluting coronary stents in swine26 in which early inhibition of restenosis at 30 days was followed by accelerated neointima formation and a paradoxical increase in the severity of restenosis by 90 and 120 days after stent implantation. There are important differences between the mechanisms underlying restenosis and vein graft disease that limit direct comparison, however. First, the stent study failed to distinguish between the effect of the drug rapamycin and that of the vehicle. Our data suggest that rapamycin toxicity is the most likely explanation for our findings. Second, promotion of restenosis in the stent study was attributed to peristrut inflammation that was more marked in drug-eluting as opposed to non–drug-eluting stents. As coronary stents are applied intraluminally, peristrut inflammation directly affects neointimal thickness. Conversely, in the present study, inflammation occurred only in the vicinity of the non–drug-eluting microspheres in the vein adventitia, which we have shown have no effect on the progression of vein graft disease. Third, although neointimal hyperplasia is a prominent feature of both lesions, positive remodeling, which preserves luminal area in the presence of vein wall thickening,18 and adventitial neoangiogenesis16 are important additional determinants of vein graft disease progression. By inhibiting these processes, rapamycin promotes vein graft disease in the long-term despite its early antiproliferative effects. Our results do not discount the possibility that lower rapamycin doses with longer elution times may avoid local toxicity. They do caution, however, against the introduction of antiproliferative agents for the prevention of vein graft disease without careful consideration of the appropriate therapeutic window. Alternatively, locally applied agents with lower toxicity or that promote neoangiogenesis may have greater therapeutic potential. A final point of comparison is that although rapamycin-eluting stents seem to accelerate restenosis in swine, clinical studies have demonstrated continued efficacy at up to 4 years poststenting.26 The differences in long-term efficacy of rapamycin-eluting stents between humans and swine may reflect differences in the baseline vessels; human vessels have underlying advanced atherosclerosis, but there also may be important species differences. We cannot discount that these differences also may apply to locally eluted rapamycin in vein grafts, but we believe that the toxicity that we have demonstrated precludes any clinical evaluation of this technique.

In conclusion, sustained-release rapamycin from periadventitial PVA microspheres inhibits vein graft thickening in porcine saphenous vein-to-carotid artery interposition grafts after 4 weeks. This effect is not sustained up to 12 weeks. The potential clinical applicability of slow-release rapamycin may be limited by a high incidence of vein graft complications that appear to arise from local toxicity and are more marked at higher doses.

Acknowledgments

We thank Dr Ray Bush and Ms Marcie Wyatt for their assistance with the experimental models.

Sources of Support

This study was supported by a project grant from the British Heart Foundation. Mr Murphy is funded by the Higher Education Funding Council for England. Ms Hua Lin is supported by the NIHR Bristol Biomedical Research Unit in Cardiovascular Medicine.

Disclosures

None.

References

The long-term success of vein bypass grafts to the coronary arteries is limited by accelerated atherosclerosis that leads to graft thrombosis in up to 50% of vein grafts within 10 to 12 years and can lead to recurrent angina, ischemic syndromes, and the need for repeat revascularization or death. There is no effective treatment, and vein graft disease remains a clinical problem that affects millions of patients worldwide. The recent clinical success of drug-eluting intracoronary stents that reduce neointima formation and restenosis rates and the prominent role of neointima formation in vein graft disease have led several investigators to assess whether a similar strategy might be effective in vein grafts. We and others have shown that locally applied antiproliferative drugs, including rapamycin, inhibit vein graft disease but only in the short term. In this study, we evaluated periadventitial rapamycin-eluting microspheres to assess whether prolonged exposure to rapamycin may have long-term benefits, and the results were not successful. Prolonged rapamycin exposure caused significant local toxicity with high rates of early graft rupture and late acceleration of vein graft disease in those grafts that remained viable. Altered drug-release characteristics may have produced a different outcome; however, the high level of local toxicity that we have shown means that clinical translation of this technique will be difficult.

The effects of therapeutic agents on processes such as angiogenesis or vessel remodeling are additional considerations beyond the inhibition of neointimal formation for the long-term prevention of vein graft disease.

**CLINICAL PERSPECTIVE**


Periadventitial Rapamycin-Eluting Microbeads Promote Vein Graft Disease in Long-Term Pig Vein-Into-Artery Interposition Grafts
Thirumaran Rajathurai, S. Imran Rizvi, Hua Lin, Gianni D. Angelini, Andrew C. Newby and Gavin J. Murphy

Circ Cardiovasc Interv. published online March 23, 2010;
Circulation: Cardiovascular Interventions is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2010 American Heart Association, Inc. All rights reserved.
Print ISSN: 1941-7640. Online ISSN: 1941-7632

The online version of this article, along with updated information and services, is located on the World Wide Web at:
http://circinterventions.ahajournals.org/content/early/2010/03/23/CIRCINTERVENTIONS.109.864660

Data Supplement (unedited) at:
http://circinterventions.ahajournals.org/content/suppl/2010/04/19/CIRCINTERVENTIONS.109.864660.DC1

Permissions: Requests for permissions to reproduce figures, tables, or portions of articles originally published in Circulation: Cardiovascular Interventions can be obtained via RightsLink, a service of the Copyright Clearance Center, not the Editorial Office. Once the online version of the published article for which permission is being requested is located, click Request Permissions in the middle column of the Web page under Services. Further information about this process is available in the Permissions and Rights Question and Answer document.

Reprints: Information about reprints can be found online at:
http://www.lww.com/reprints

Subscriptions: Information about subscribing to Circulation: Cardiovascular Interventions is online at:
http://circinterventions.ahajournals.org//subscriptions/
Supplemental Material

**eFigure 1.** Graphs showing A. Lumen radius/wall thickness ratios B. Endothelialisation, C. Apoptosis (Cleaved Caspase 3 Staining) and D. Inflammatory (MAC 287 positive staining) cells in experimental porcine vein grafts. Bars represent mean (S.E.M).

**eFigure 2.** Graphs showing A. weight gain, B. renal function and C. liver enzyme release in pigs receiving rapamycin eluting microbeads.
Re-endothelialisation in Porcine Vein Grafts

Time (weeks)

0 0.5 1 1.5 2 2.5 3 3.5 4

Enothelial Score

Low Dose Control  Low Dose Treatment  High Dose Control  High Dose Treatment

Apoptosis in Day 7 Vein Grafts

Time (weeks)

0 2 4 6 8 10

Percentage

60µg.cm-2 120µg.cm-2

Dose

Adventitial Inflammation in 4 Week Porcine Vein Grafts

Mean Cell Count per x40 Field

0 0.5 1 1.5 2

60µg.cm-2 120µg.cm-2

Dose

Radius/Wall Thickness Ratio

0 20 40 60 80 100 120 140 160

Ratio

Low Dose Control  Low Dose Treat  High Dose Control  High Dose Treat

Adventitial Inflammation in 4 Week Porcine Vein Grafts
**Measured Weights in High and Low Dose Pigs**

- **High Dose**
- **Low Dose**

**Serum Creatinine in High and Low Dose Pigs**

- **Low Dose**
- **High Dose**

**Serum Alanine Aminotransferase in High and Low Dose Pigs**

- **Low Dose**
- **High Dose**

---

**Figure 2**